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Carcinogenic Substances in Human Tissues

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Some of the problems presented by the carcinogenic substances that have been extracted from human tissues will be solved only when these compounds have been chemically identified and assayed. At the present time very difficult questions have been raised by the fact that the substances have been found in the tissues from some patients dead of cancer but not in others, and further, in the tissues of some subjects dead of diseases other than cancer, and again not in others.

The admirable work of Steiner (13, 14, 15) shows that about 20 per cent of the livers of human subjects who died of cancer or some other cause contain carcinogenic substances. This result disposes of any simple or direct relation between the presence of the substances and the incidence of cancer in the subject. Of particular value is Steiner's work in examining each tissue extract separately using a separate batch of experimental animals. Thirty-seven livers from cancer patients yielded 8 active extracts (22 per cent) while 6 of 30 livers from noncancer patients gave active extracts (20 per cent). This is to say that, in a sample of 67 human livers, 1 in 5 contain detectable carcinogens.

It would not be very satisfactory to "explain" on a fortunate chance encounter of "1 in 5" why Schabad (9, 10), who was the first to open this line of investigation, found a benzene extract of a noncancerous liver to be inactive while the benzene extract of a cancerous liver was active, or why Sannié, Truhaut and Guérin (12) found the combined unsaponifiable fraction of 2 cancerous livers to be active but the unsaponifiable fraction of 2 noncancerous livers to be inactive. But it might explain results obtained in this laboratory (8) where the unsaponifiable fractions of some livers of cancer subjects were highly active[1] while similar preparations from other cancer livers were inactive. Other possible explanations will be referred to later. The essential features of the experiments of these workers and of Menke (11) have been collected in the tables below, (Tables I to V).

TABLE I: RESULTS OF EXPERIMENTS OF KLEINENBERG, NEUFACH AND SCHABAD (9, 10)

Material used	No. of mice	Initial 8 months	Tumor site	
			Injection	Other
1. Benzol extract of 41 cancer livers *	179	108	4	62
2. Benzol extract of 26 noncancerous livers	91	54	0	13
3. Control	634	389	0	40
4. Unsaponifiable fraction of 9 cancer livers	x † 27	13	1	3
	Rv 23	16	0	5
Benzol extract of lung (unsap.?) ‡				
5. 19 Cancer cases	Rv 67	46	1	25
	x 28	15	0	3
6. 20 Noncancer cases	Rv 44	26	0	6
	x 73	32	0	4

* In this and subsequent tables "cancer liver" denotes liver from subjects dead of cancer of any tissue or organ other than liver. "Noncancer liver" denotes liver from subjects dead of any cause other than cancer.

† x and Rv represent two different strains of mice.

‡ It is not clear in the text whether the unsaponifiable fraction or the crude extract was used.

[1] In one experiment 8 sarcomas at the site of injection developed in 11 mice which survived 11 months.

Extracts of the livers of cancerous and of noncancerous Bantu natives have been found by des Ligneris (5) in Johannesburg to be carcinogenic. He obtained 25 tumors, 7 of them malignant in some hundreds of mice by painting the skin with a solution of the unsaponifiable fraction of the livers. The unsaponifiable fraction of livers of cancerous or noncancerous Europeans did not produce skin tumors in mice.

Aptekman, King and Lewis (2) used a benzene extract of a rat tumor which had been produced by a carcinogenic hydrocarbon and had been grafted through more than 20 generations. They obtained 5 sarcomas in 28 rats and also 2 sarcomas in 24 rats injected with a benzene extract of the livers of the original rats which bore the grafted tumors.

Menke's results in his third experiment are difficult to explain except on the assumption that the extractable fat from the breast diluted the extract from the tumor, or that the still normal part of the breast contained factors inhibitory to carcinogenic action, or recourse must be made to the random distribution observations of Steiner.

Remote tumors.—A number of workers have given attention to the tumors arising at sites other than at the place of injection; here again Steiner's results, in opposition to those of Schabad and Sannié and their co-workers, show little difference in tumor production between preparations from the livers of cancerous and those from noncancerous subjects, and also surprisingly little difference between preparations that were

TABLE II: RESULTS OF EXPERIMENTS OF STEINER (13-15)

Material used	Sarcomas at injection site	
1. Unsap. of 8 cancer livers (pooled)	12 in 56 mice	14/30 *
2. Unsap. of 7 noncancer livers (pooled)	5 " 63 "	17/54
3. Unsap. of 37 cancer livers (tested separately); 8 were found active	12 " 456 "	274 (12 ms.)
4. Unsap. of 30 noncancer livers (tested separately); 6 were found active	10 " 440 "	277 (12 ms.)
5. Benzene extract of a cancer liver	0 " 32 "	16/26
6. Benzene extract of 11 cancer livers		
A. Ether soluble fraction	0 " 15 "	10/14
B. Ether insoluble fraction	0 " 67 "	41/66
C. Unsap. fraction of A.	0 " 17 "	12/17
7. Unsap. of 4 human cancers	0 " 45 "	32/42
8. Unsap. of 10 human cancers—large part of cholesterol removed by crystallization	0 " 35 "	7/35
9. Sesame oil control	0 " 18 "	—
		Survivors after 12 ms.
10. Heated sesame oil (350° C.)	3 " 31 "	9
11. Fat from overfried meat	0 " 20 "	19
12. Unsap. from 11	0 " 10 "	8
13. Same meat as 11 but excess fat removed by acetone extraction, residue extracted with benzene; benzene extract	1 " 20 "	18
14. Unsap. fraction from 13	0 " 8 "	7
15. Numbers 1 and 2 into rats, no tumors		

* Fractions: Numerator = tumors of lung, lymphatic tissue, mamma, uterus, ovary or liver, in survivors. Denominator = survivors at 6 months.

TABLE III: RESULTS OF EXPERIMENTS OF SANNIÉ, TRUHAUT AND GUÉRIN (12)

Material used	Mice: Initial 12 months		Tumors at injection site: Sarcoma	Hemorrhagic cyst	Other sites
1. Unsap. of 2 cancer livers	25	20	6	12	6
2. Unsap. of 2 noncancer livers	25	20	0	5	3
3. Olive oil control	25	15	0	0	1

TABLE IV: RESULTS OF EXPERIMENTS OF HIEGER (8)

	Mice: Initial	1 year	Sarcomas: At site of injection
1. Unsap. of 3 cancer livers	28	17	11
2. Unsap. of Bantu livers *	30	19	5
3. Ether extract of 2 cancer livers	37 (baby mice)	14	2
4. " " " " " "	17 (not baby mice)	7	0
5. Benzene extract of 4 cancer livers	60	15	0
6. Unsap. of 3 cancer livers	27	18	0
7. Unsap. of 3 noncancer livers	40	19	0
8. Lard (*i.e.*, solvent) controls	303	142	0

* For this material which was sent by air I am indebted to Dr. des Ligneris of the South African Institute for Medical Research.

active in producing local sarcoma and those which were inactive.

Effect of saponification.—Schabad, and also Menke, find that a simple extraction of tissue with fat solvents gives an active preparation, while saponification which removes, at any rate in the case of liver, nine-tenths of the total extractable "fat" fails to give a product of increased potency. Thus no hint is given whether the agent belongs to the fatty glycerides and phospholipins or to the sterols. Steiner on the other hand found that in one series of experiments 15 livers pooled in two groups of 7 and 8 gave strongly carcinogenic unsaponifiable fractions, but the benzene extracts of 11 pooled cancer livers were inactive. It would be very curious if the first two groups (7 and 8 livers) happened to contain one or more of the active livers which occurs once in 5 while the third group of 11 livers did not contain a single "1 in 5" liver. Alternative explanations would be that saponification concentrated the agent by removing inactive fats, or that the active agent is an artifact.

Steiner's preparations were obtained by somewhat drastic means, namely saponification with potash at the boiling point for 18 hours. Such treatment might conceivably form a carcinogen from a precursor or might destroy a carcinogen already present, but since all of the 67 livers were treated in the same way the evidence is in favor of a very stable carcinogen, present infrequently (namely 1 in 5). It may be possible in the future to apply less drastic methods to the analysis of tissue fats, such as chromatography or the centrifugal molecular still.

In experiments carried out here, 2 sarcomas were produced in 37 mice injected from the second day after birth with an ether extract of cancer liver. (The same extract injected in 17 mice 7 weeks old produced no tumors.) Menke and Steiner carried out the obvious test of using the same preparation for making (a) the simple extract and (b) the unsaponifiable fraction. In Menke's experiments and in one of Steiner's neither (a) nor (b) was active, but in Steiner's overfried meat experiment (No. 14) the unsaponifiable fraction was inactive but the simple benzene extract produced one sarcoma. Thus, while Steiner and Hieger showed that saponification nearly always gives products of higher potency than does simple extraction, one critical test (based on a single positive result) suggests the reverse. Menke tries to compromise by proposing that there are two agents, one in the saponifiable part and one in the unsaponifiable.

Minimum number of mice.—It may be noted that much depended upon the single sarcoma just mentioned. How often does one see the bald announcement without comment of a single sarcoma being produced by a tissue extract or a sterol derivative? Such results emphasize the sensitiveness of one particular animal rather than the carcinogenic potency of a material under test. The assessment of the activity of materials is satisfactory in proportion to their potency. In this kind of work the assumption is made that the animals used for testing have uniform susceptibility distribution yet everyone knows this to be far from the case.

In the papers of Bryan and Shimkin (3, 4) evidence is given that even in pure strains of mice great variations become obvious when the stimulus is near the threshold of potency (Table VI).

It will be seen from the tables that 0.005 mgm. 1,2,5,6-dibenzanthracene gave tumors in 4 per cent of animals in tests by Dobrovolskaia-Zavadskaia, in 8 to 9 per cent in tests by Bryan and Shimkin and was inactive in the tests by Lettinga. Even less satisfactory is the result with benzpyrene, where 2 mice out of 81 responded to 0.00195 mgm., but 40 did not respond to a dose 4 times as great, 19 mice to one 8 times and 16 to a dose 16 times. How many mice are necessary to ensure that a proportion of the very sensitive individuals are present in any group of animals? In other

TABLE V: RESULTS OF EXPERIMENTS OF MENKE (11)

Material used	(a) (Soxhlet extraction) 4 solvents	(b) Unsap. of (a)	(c) Digitonide fraction	(d) Non-digitonide fraction	(e) Non-carbonyl fraction
1. Breast cancer, tumor alone; 3 extracts from 3 tumors	7/36 *	—	—	—	—
2. Cancerous breast after removal of tumor; 2 extracts from 2 breasts	0/33	0/18	0/6 †	0/6 †	—
3. Breast cancer whole, *i.e.*, breast + tumor; 2 cases	0/18	0/22	—	—	0/18 †
4. Noncancerous breast; 4 cases	0/54	0/36	—	—	—
5. Rectal cancer	0/15	0/6	—	—	—
6. Osteogenic sarcoma (femur)	1/12	—	—	—	—

* Fractions: Numerator = number of sarcomas produced. Denominator = number of mice.
† Made from the extract of a separate breast, not that used for (a) and (b).

TABLE VI: VARIATIONS IN RESPONSE OF MICE TO CARCINOGENS

Investigators and materials used		No. of mice	Tumor mice	Tumors per cent	Mean latent period, months
DOBROVOLSKAIA-ZAVADSKAIA					
1,2,5,6-Dibenzanthracene					
.01	mgm.	328	37	11	
.005	"	364	13	4	
.0025	"	167	2	1.2	
.00125	"	158	0	0	
LETTINGA					
1,2,5,6-Dibenzanthracene					
5.0	mgm.	10	5	50	4.3
2.5	"	10	8	80	3.8
1.0	"	10	9	90	3.6
0.5	"	10	10	100	3.6
0.25	"	30	26	90	4.6
0.125	"	20	9	45	6.0
0.05	"	10	4	40	7.3
0.0125	"	20	4	20	8.0
0.005	"	20	0	0	—
BRYAN AND SHIMKIN (3, 4) *					
Methylcholanthrene					
1.0	mgm.	20	20	100	2.4
0.5	"	21	21	100	2.6
0.25	"	21	21	100	2.8
0.125	"	21	21	100	3.3
0.062	"	21	17	80	3.9
0.031	"	20	13	65	5.2
0.0156	"	18	6	33	4.6
0.0078	"	17	3	18	7.0
0.0039	"	19	0		
1,2,5,6-Dibenzanthracene					
8	mgm.	21	16	76	3.8
4	"	20	17	85	3.8
2	"	19	19	100	3.7
1	"	22	22	100	3.6
0.5	"	21	20	95	3.8
0.25	"	21	19	90	4.0
0.125	"	23	21	91	4.5
0.062	"	20	20	100	5.1
0.03	"	21	16	76	6.3
0.0156	"	19	6	32	6.0
0.0078	"	40	6	15	8.8
0.00195	"	79	2	2.5	9.5
3,4-Benzpyrene					
8	mgm.	21	20	95	3
4	"	19	16	84	3
2	"	19	19	100	3
1	"	20	18	90	3.3
0.5	"	19	19	100	3.9
0.25	"	21	14	67	4.4
0.125	"	19	15	79	5.1
0.062	"	20	4	20	5.8
0.031	"	16	0	0	—
0.0156	"	19	0	0	—
0.0078	"	40	0	0	—
0.00195	"	81	2	2.5	8.4

* C3H mice were used by these investigators.

words, how is the sensitivity distributed? These questions are certainly in need of investigation.

Furthermore the survival rate during the course of the tests adds an additional source of complexity in assessing the potency of material. It would be expected that those series yield tumors where the mice live exceptionally well and therefore are exposed to the material for the longest time and indeed some evidence for this view is afforded by the writer's experience (Table VIII).

The survival rate of the positive series would have been further improved if the mice that did develop tumors had not been killed prematurely. However, a number of animals in Steiner's series that lived exceptionally well produced no tumors.

The data from Bryan and Shimkin's papers (3, 4) show that the hydrocarbons even at threshold dilution produce tumors in about 9 months, yet tissue carcinogens often require a latent period of about 18 months (see Tables VI and VII), suggesting that different processes are involved in carcinogenesis by the two types of agent. Experiments where dilutions of feeble carcinogens like 3,4-benzphenanthrene were used would give results of the greatest interest, especially since a prolonged latent period is characteristic of most human spontaneous cancer.

THE POSSIBILITY OF CONTAMINATION

Atmospheric contamination, *i.e.,* benzpyrene in soot, as a possible factor in the preparation of active fractions from tissues must be considered. Very little benzpyrene is required for sarcoma production (of the order of from 50 to 2 μgm.) and although such a quantity is not very likely to be found in the soot dust which is deposited on the materials, solvent and apparatus during an experiment, nevertheless further investigation on this matter should give interesting results. For example, some nonfluorescing benzene was exposed in a beaker to the air near this Institute, which is situated in a smoky part of London. After about an hour the benzene was tested in ultraviolet light and it was found not only that the solution became fluorescent but this fluorescence gave distinctly the spectrum bands of 3,4-benzpyrene.

The search for carcinogens in liver was pursued here during 1937, 1938 and 1939 in the building where the carcinogenic hydrocarbons were discovered and first synthesized, and in the very laboratory (see footnote 1) where potent crystalline preparations of 3,4 benzpyrene were first elaborated from pitch in 1930-32. In 1939 we moved to a fresh building 200 yards from the old laboratories, and the first two extracts tested, *i.e.,* the unsaponifiable fraction of 3 cancer and of 3 noncancer livers, gave negative results.

TABLE VII: TISSUE CARCINOGENS, LATENT PERIOD IN MONTHS

Figures represent new tumors

Investigators, and tissues used by them	Months 5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Total
Steiner																											
Cancer livers (8 pooled livers)			1	3	2	1	1	2	2																		12
Noncancer livers (7 pooled livers)								1	1	1				1			1										5
Cancer livers (37 livers tested separately)		1						4			4			2												1	12
Noncancer livers (30 livers tested separately)					1			1			3			1			2		1							1	10
Kleinenberg, Neufach, and Schabad		1				2							1														4
Sannié, Truhaut, and Guérin									1	1			1	1	1		1										6
Hieger																											
Table IV						1	2	2	1		1		1	2	2	1	2	1	2								18
Tables X and XI										1	2	1			1	1			1	1							8
																											75

TABLE VIII: SURVIVAL TIME IN RELATION TO TUMOR PRODUCTION

	Surviving mice 1 +ve series *	2 −ve series †	3 Lard control ‡
No. of mice at start	73	329	100
Percentage at start	100	100	100
" " 10th month	76	48	62
" " 11th "	70	44	59
" " 12th "	63	39	59
" " 13th "	54	33	45
" " 14th "	52	29	20
" " 15th "	44	22	14
" " 16th "	38	18	6.6
" " 17th "	33	14	6.6
" " 18th "	26	10	6.6
" " 19th "	18	8	6.6
" " 20th "	12	6.4	4.4
" " 21st "	10	4.6	4.4
" " 22nd "	4	2.7	4.4
" " 23rd "	1.7	—	4.4
" " 24th "	—	—	3.3

* Series 1. Mice injected with liver extracts where tumors were produced.

† Series 2. Mice injected with liver extracts where tumors were not produced.

‡ Series 3. Controls using solvent alone. This batch of 100 mice were living at approximately the same time as series 1 and 2. They represent the first 100 of the total controls (303 mice) in Table IV. The survival rate at 12 months is in both groups surprisingly similar (59% and 47%) considering the numerous disturbing factors which might have produced a different result.

The identification of the carcinogen in liver will be necessary in order to exclude the possibility of accidental contamination.

It is not intended to convey the impression that aerial contamination by benzpyrene alone can explain the positive results with tissue fractions. Contamination by benzpyrene may be one of the factors involved but there is no evidence that any such contamination occurs. None of the other workers in this field, whose results are tabulated above, have ever suggested that contamination occurred in their experiments, and one has of course no evidence of any such possibility. Some evidence can be adduced in favor of the opposite view; thus some liver preparations made in the old laboratory were inactive. A somewhat similar problem presented itself to Earle (7) who found that in experiments on carcinogenesis in tissue cultures the controls for some reason also showed carcinogenic changes and he concluded that contamination with methylcholanthrene had occurred. He states, "The laboratories in which this work was carried out are in the same building with other laboratories and animal rooms where large amounts of methylcholanthrene and similar carcinogens were handled. The design of the air conditioning and heating system of the building is such that there has been some trace recirculation of used air from many rooms, in some of which the carcinogen was certainly handled. Because of insufficient knowledge of the activity of low concentrations of methylcholanthrene and other carcinogens in tissue culture, the hazard of such trace contamination from this source also cannot be evaluated at present, although it is felt that its possible significance must not be overlooked. . . . As the most probable explanation of the alterations in the control cultures and as a working basis for further study, the hypothesis is advanced that a trace contamination of methylcholanthrene occurred in spite of all precautions taken."

It may be recalled that Liebermann after having

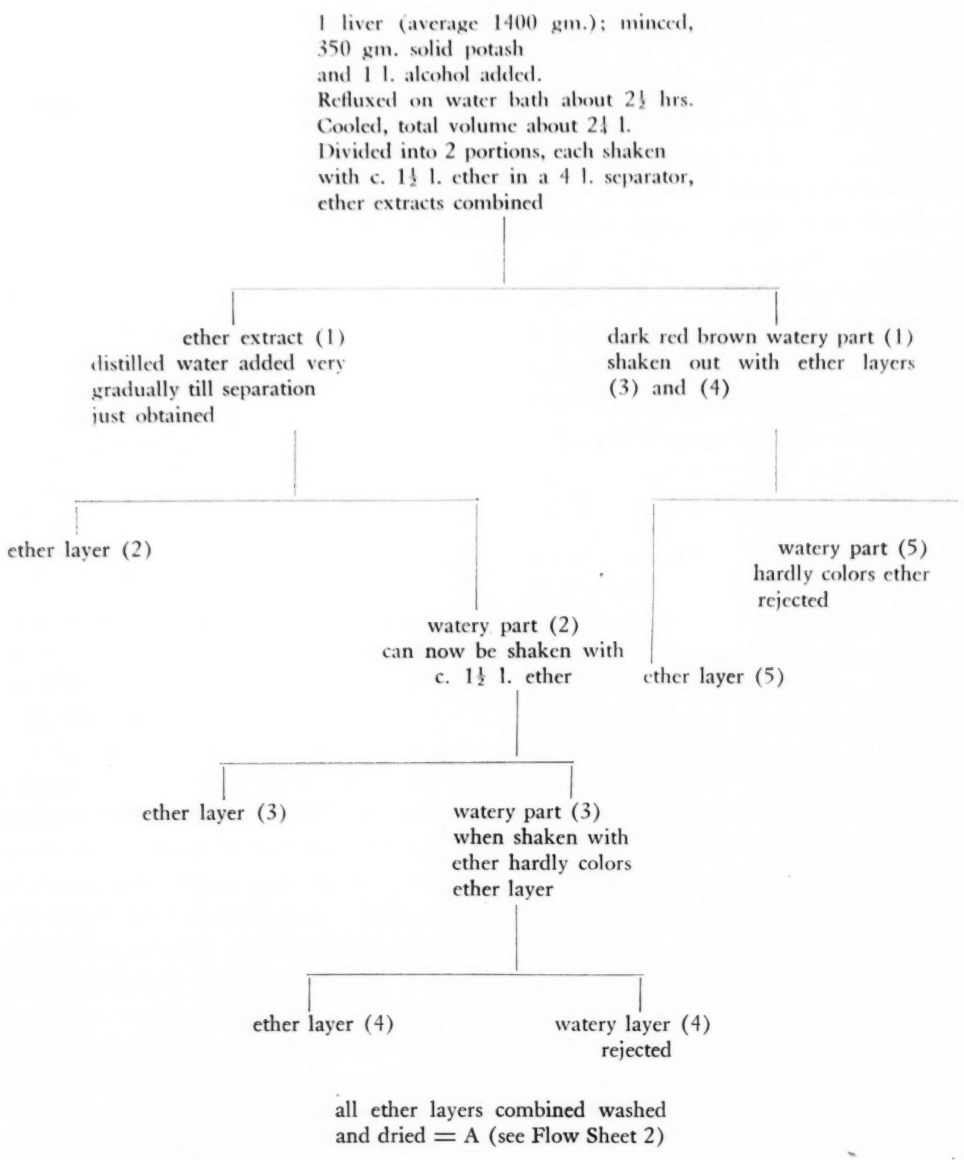
once prepared a particular crystalline modification of cinnamic acid was unable to prepare alternative forms since enough molecules of the first variety were left in the laboratory to seed his solutions.

The experimental bacteriologist can at least sterilize his apparatus and material. How much more difficult is the task of those who wish to detect minute traces of carcinogens.

Fractionation of Unsaponifiable Material from Tissues

Technic (*a*).—Although the first 6 liver preparations tested in the new building of this Institute failed to produce tumors, experiments were set going on the fractionation of the crude unsaponifiable material. Owing to the very long latent period before tumors appear in this work it was necessary to start new experiments long before information from previous experiments was available. This plan was justified later in the light of Steiner's results, for even if a considerable proportion of the livers did not contain detectable carcinogen yet there might be an active liver among them if a sufficiently large number were worked up together. Then by fractionating the pooled unsaponifiable fraction, either a fraction might be obtained containing the larger part of the active substance or it might even be possible to identify the compound itself. In either case a knowledge of the physical and chemical properties of the active fraction or compound would have obvious advantages since its identification and estimation by physicochemical means in any particular tissue might require only a few days' work in-

Flow Sheet 1



FLOW SHEET 2

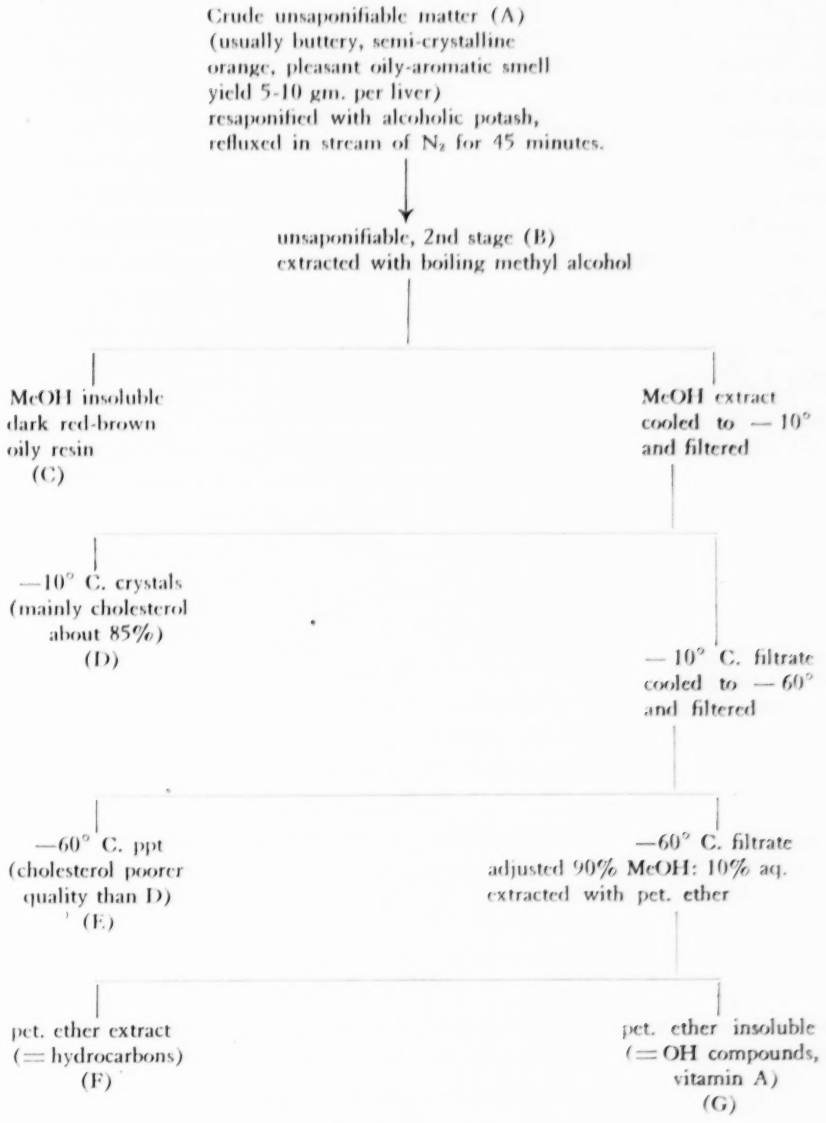


TABLE IX: FRACTIONATION OF UNSAPONIFIABLE MATERIAL

	Fraction	Noncancer livers	Cancer livers	Noncancer lung-kidney-muscle	Cancer lung-kidney-muscle	
No. of human subjects		4	4	16	10	
Weight of tissue		c. 6 kgm.	c. 6 kgm.	c. 34 kgm.	c. 19 kgm.	
Crude unsap.	A	22 gm.	c. 30 gm.	72 gm.	72 gm.	
After 2nd sapon.	B		19 "	40 gm. from 50 gm. A	30 gm. (from 34 gm. A)	23 gm. from 28 gm. A
MeOH insoluble	C		3 "	trace " "	small amount weight unknown	2 " " "
— 10° Crystals	D	8 "	8 "	22 gm. " "	15 gm. (from 34 gm. A)	13 " " "
— 60° ppt.	E	3 "	2 "	3 " " "	4 " " "	3 " " "
Pet. ether soluble	F	2 "	1 "	2 " " "	1 " " "	1.5 " " "
90% MeOH "	G	—	0.25 "	0.6 " " "	0.6 " " "	0.5 " " "

stead of an animal test lasting 1 or 2 years and provided, as unfortunately does not always occur, that sufficient mice survived the long latent period.

The unsaponifiable matter from liver was first fractionated by a much simplified form of the technic used in preparing Vitamin A concentrates from liver. It was suggested by Dr. Mead of the British Drug Houses, Ltd., to whom we are much indebted. The steps are indicated in the two Flow Sheets and in Table IX.

Soon after this work had been begun Schabad reported the production of a tumor in a mouse with an extract not of liver but of the lung of a subject dead of cancer. In the experiments carried out here it was decided that in order to obtain enough material for working up the unsaponifiable fraction, a mixture of the larger organs and tissues other than liver might be used. Lungs, kidneys and skeletal muscle were removed at necropsy and treated together in the same way as liver.

Little attention was given to the particular type of cancer involved. The immediate aim was to find the physicochemical nature of the active substance although the possibility remained that different tissues might contain different carcinogens. Noncancer tissues were from elderly or middle aged subjects dead of any cause other than cancer.

The livers or other tissues were placed in the cold room as soon as possible after removal from the body. They were usually saponified on the next day but sometimes 2 or 3 days elapsed between death and the working up of the tissues, by which time they were usually frozen solid. The survival rate of the active substance in dead tissue is unknown but a compound which will withstand boiling alcoholic caustic potash for 3 hours is not very likely to be decomposed by tissue enzymes near freezing point.

Some of Steiner's active preparations survived 18 hours' boiling with potash. Fatty esters can however be saponified at room temperature by the use of sodium ethoxide in a mixture of alcohol and ether and of course the avoidance of powerful reagents is desirable. Hence experiments have been begun where livers are saponified at room temperature for 18 hours then for 3 to 4 hours at 45° C. The second saponification consists of 30 minutes' refluxing in a stream of N_2 with alcoholic potash.

Controls.—1. Control experiments where the solvent (lard) alone is injected have already been referred to. No tumors have been obtained in some hundreds of mice which have been treated by exactly the same technic as is used for tissue fractions.

2. Tests are in progress to see if carcinogens are produced as artifacts during saponification, since two workers (Steiner and Hieger) found that activity was increased by this process, or more exactly the unsaponifiable fraction of livers has proved much more carcinogenic than crude fatty extracts. Alcohol, and especially industrial spirit, contains traces of acetaldehyde which is resinified by alkali and this resin, being soluble in ether, would be found in the unsaponifiable fraction. In three experiments, distilled spirit, distilled alcohol and alcohol to which acetaldehyde had been purposely added were refluxed with potash for 3 hours in quantities adjusted to imitate the conditions of liver saponification. The mouse test on the products has now been in progress for 20 months.

3. Cholesterol undergoes oxidation in alkaline solution. 10 gm. of cholesterol obtained from British Drug Houses, Ltd., *i. e.,* about twice as much as contained in a human liver, was refluxed with potash and (a) alcohol and (b) spirit in a control experiment, no liver tissue being present. The mouse test on the products has now been in progress for 14 months.

4. The active fraction (D, Flow Sheet 2) consists largely of cholesterol. Commercial cholesterol was therefore injected into mice in suspension in lard of 25 per cent concentration. One hundred and thirty-two mice of 5 different strains have been treated and, although many died early in the experiment, a single C57 mouse developed a typical spindle cell sarcoma at the site of injection in 11 months. This tumor has now reached its 16th generation and grows with unusual speed for, if allowed, it will become as large as the mouse in a month.

Technic (*b*).—No polycyclic hydrocarbons were allowed in the laboratory. As has been pointed out earlier, some aerial contamination by soot or other carcinogenic substances was practically unavoidable owing to the position of the Institute and the laboratory. Where practicable, apparatus and materials were protected from atmospheric dust by the plentiful use of clean paper covers during the experiments. Glassware and other apparatus was cleaned with household abrasive, alcoholic potash, H_2SO_4 conc., alcohol, ether and finally distilled ether. Solvents were distilled over eosin which could be detected in the distillate by ultraviolet fluorescence if traces were sprayed over. It was found that a fractionating column has to be lightly stuffed with glass wool before the invisible spray of distilland can be stopped. Solid reagents were not to be so easily purified; they were used at analytical reagent quality and washed with distilled ether when required. Nevertheless all such precautions were obviously far from perfect.

Injection.—As far as possible the fractions were injected in solution in lard at 15 per cent concentration except where little of a fraction was available and economy had to be exercised. Injections were made subcutaneously in the flank, either 0.1 cc. or 0.2 cc. fortnightly, the object being to maintain a reservoir of material which could be felt through the skin as a soft nodule. In some cases, after 5 or 6 injections no further injection was needed for a year or more; in other cases the reservoir had to be replenished more frequently.

Experimental mice.—Unless otherwise stated, the mice used in all the work described in Tables IV, IX

and X and for testing the fractions in Flow Sheet 2 were mixed commercial stock of unknown heredity obtained from the same source. In the middle of the war these mice began to suffer serious epidemic losses, and the same befell strains obtained from the Lister Institute and the Medical Research Council. C3H mice, although highly sensitive to carcinogens applied subcutaneously (1), seem to be easily poisoned by the fractions; moreover the females are lost early owing to mammary carcinoma.

In the search for some more resistant strain the C57 blacks were bred here and used extensively. These mice, which are placed fourth in order of sensitivity to carcinogenic hydrocarbons by Andervont, have now been used here for over 2 years but they do not give the impression of being remarkably sensitive to tissue carcinogens. Tests with the original fractions that gave sarcomas in ordinary stock have now been in progress for 22 months without result. Of late the original mixed stock have been bred in the laboratory while excluding all outside sources, have developed into a race of large, beautifully healthy animals, and are now being tested with the tissue fractions.

Sarcoma Production

The control series receiving lard only have so far given negative results but some of the tests are still in progress. The results obtained with the various fractions of unsaponifiable material are given below (Tables X and XI). The tumors were spindle cell sarcomas at the site of injection. The negative result with fraction D of noncancer liver is noteworthy and this test will be repeated. With 1 exception the tumors (7 out of 8) occurred in the series injected with fraction D (see Flow Sheet 2) irrespective of its origin, *i.e.*, whether the tissue was liver or lung-kidney-muscle or whether the tissue was from cancerous or noncancerous subjects. Moreover the activity of the 3 fractions was of the same order but the precise significance of this similarity is unknown for the conditions of the test may be such that large relative differences in agent do not effect proportionate differences in potency.

Cholesterol estimations on fractions D, from different sources, by digitonin precipitation give figures ranging from 74 to 92 per cent cholesterol, which are probably rather high since the digitonin method does

Table X: Sarcoma Production by Subfractions of Unsaponifiable Fraction

Tissue	Fraction (see Flow Sheet 2)	Surviving mice (months) 0	3	6	9	12	15	18	21	24	27	30	33	36	Tumors
Noncancer															
Liver	D	20	15	12	11	8	6	3	3	1	0				
	E	15	9	7	5	5	3	2	2	0					
	F	15	10	10	9	6	6	6	3	1	0				
Lung-kidney-muscle	B	16	16	16	15	12	10	5	3 *	0					1
	D [1]	25	15	15	14	14	8 *	2	1 *	0					2
	E	10	10	10	10	10	8	6	3	1	1	0			
	F	10	10	10	10	10	9	7	4	1	0				
	G	5	5	5	5	5	5	4	1	1	0				
Cancer															
Liver	C	10	10	9	9	8	6	4	3	2	0				
	D	10	10	10	10	9	6 *	4 *	3	3	0				2
	E	10	8	8	8	8	6	3	3	0					
	F	10	10	9	7	6	4	4	2	0					
Lung-kidney-muscle	B	15	15	15	15	14	11	6	3	2	1	0			
	C [3]	10	9	8	8	0									
	D [2]	25	16	15	14	10 **	9	4 *	0						3
	E	10	10	10	10	9	9	7	4	1	1	1	0		
	F	10	9	9	6	6	6	6	1	0					
	G	5	5	5	4	3	3	2	2	0					

* Appearance of tumor.

** Mice were of mixed stock.

[1] Being tested on 15 C57 mice also; 8 survived 12 months.

[2] Being tested on 9 C57 mice also; 7 " " "

[3] Being tested on 5 C57 × C3H hybrids; 0 survived 12 months.

C57 mice are, however, not entirely resistant to cholesterol-rich fractions, for 3 sarcomas have been obtained in experiments which will be described in a forthcoming publication.

not distinguish between cholesterol and other 3(β)-hydroxysteroids. Dr. Shoppee (personal communication) working with a large sample (30 gm.) of D-fraction finds a cholesterol content of 85 per cent based on the isolation of chromatographically pure cholesteryl acetate, m.p. 115° $[\alpha]\frac{20}{D} = -43.6° \pm 1°$.

This fraction, D, will be considered then to have a cholesterol content of about 85 per cent; it contained no saponifiable material and was a somewhat waxy crystalline powder, pale straw or orange colored, and obviously not homogeneous, for the colored part seemed to be unevenly adsorbed on the white part. It remains to be determined whether the active substance is present in small amount adsorbed onto the cholesterol during crystallization, or whether along with the cholesterol are crystallized appreciable amounts of allied

TABLE XI: SARCOMA PRODUCTION WITH CRYSTALLINE, CHOLESTEROL-RICH FRACTION D

Tissue	Mice at 0 months	Mice at 12 months	Tumors: Number	Tumors: Latent period, months
Noncancer				
Lung-kidney-muscle	25	14	2	15, 24
Cancer				
Lung-kidney-muscle	25	10	3	14, 15, 20
Cancer				
Liver	10	9	2	16, 19
Noncancer				
Liver	20	8	0	

sterols or sterol derivatives, among which is the factor whose identity is required. Possibly the cholesterol itself, which makes up the larger part of this fraction, is by no means inert in sarcoma production and the tumor may be the end result of the combined activity of more than one compound. Thus the carcinogenic activity of benzpyrene is increased when cholesterol is added according to the results of experiments by Dickens and Weil-Malherbe (6) where solutions of the hydrocarbon with and without cholesterol were painted on mice. The chemical constitution of the compounds associated with cholesterol contained in fraction D is being investigated by Dr. Shoppee in this Institute, who will publish his results shortly.

Subfractions of this fraction are undergoing tests in mice.

SUMMARY AND CONCLUSIONS

1. Previous work is reviewed.

2. Attention is drawn to the main difficulties in this investigation: (a) The low potency of human tissue carcinogens as indicated by the prolonged latent period. (b) The sporadic occurrence of these substances (Steiner). (c) The uncertainties with regard to "susceptibility" of the experimental mice.

3. A technic of fractionation of unsaponifiable material from tissues is described.

4. A sarcoma-producing fraction has been obtained from mixed lung-kidney-muscle of cancerous and noncancerous human subjects, and from the liver of cancerous patients.

5. In all three cases the carcinogenic substance is found in the cholesterol-rich fraction of the unsaponifiable material. This fraction is a crystalline mixture of compounds containing on the average about 85 per cent cholesterol.

ACKNOWLEDGMENT

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Multiple Peritoneal Sarcoma in Rats from Intraperitoneal Injection of Washed, Ground *Taenia* Larvae

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Published reports (1, 2, 3, 4) show that sarcoma is an almost inevitable complication of infestation of the liver or the subcutaneous tissues of rats with *Cysticercus fasciolaris,* the larva of the common tapeworm of the cat. The present experiments demonstrate that an active agent is present in washed, freshly ground parasites.

MATERIALS AND METHODS

Living parasites were removed from uninvolved cysts of rats with and without gross *Cysticercus* tumors, washed in large volumes of sterile saline, cut in fragments, ground in a mortar and suspended in saline. The saline suspension was injected into the peritoneal cavity of uninfested rats related to the hosts of the parasites and also into unrelated rats. Multiple peritoneal sarcomata and mesothelioma were observed within 23 to 787 days following injection.

RESULTS

The results are summarized briefly in Tables I and II. The data presented in Table I deal with experiments in which the larvae were obtained from uninvolved cysts of rats bearing *Cysticercus* sarcoma. The first 3 columns give the date of the experiment, the number of parasites used to make the suspension, and the number of rats which were injected. The next four columns show the number of rats which survived the minimum latent period in 2 categories, that is, those related to the hosts of the parasites from which the suspension was made and those which were unrelated to the hosts of the parasites, and in each case the numbers which were positive. The final 3 columns show the minimum, maximum and average latent period in days. In 8 separate experiments 320 parasites were used to make the saline suspension which was injected into 103 rats. Of the rats which survived the minimum latent period, 56 were related to the hosts of the parasites and 44 were unrelated. Among the related rats 51 or 91 per cent developed multiple peritoneal sarcomata 23 to 410 days after injection and 6 or 14 per cent of the unrelated rats developed peritoneal sarcomata. The average latent period of the entire group was 89 days.

The results are similarly tabulated in Table II for the experiments in which the parasites were obtained from rats showing no gross tumors. In 8 experiments, 359 parasites were used to make a saline suspension which was injected into the peritoneal cavities of 59 rats. Of the rats which survived the minimum latent

TABLE I: THE NUMBER OF RELATED AND UNRELATED RATS IN EACH EXPERIMENT WHICH DEVELOPED MULTIPLE PERITONEAL SARCOMATA FOLLOWING INTRAPERITONEAL INJECTION OF A SALINE SUSPENSION OF WASHED, GROUND *Taenia* LARVAE OBTAINED FROM UNINVOLVED CYSTS OF RATS WITH *Cysticercus* SARCOMA, AND THE MINIMUM, MAXIMUM AND AVERAGE LATENT PERIODS.

			Related rats		Unrelated rats		Latent period in days		
Date	Number of larvae	Total rats injected	Over minimum latent period	Number positive	Over minimum latent period	Number positive	Minimum	Maximum	Average
Apr. 12, 1939	10	10	3	3	7	0	59	61	60
Nov. 11, 1939	104	38	19	19	19	2	30	94	63
Jan. 26, 1940	56	16	8	6	8	1	37	94	77
Feb. 1, 1940	60	16	8	7	8	1	50	239	110
Mar. 8, 1940	32	2	2	2	0	0	23	23	23
Mar. 19, 1940	15	3	1	1	0	0	45		
July 11, 1940	14	5	3	2	2	2	99	219	147
July 21, 1944	29	13	12	11	0	0	60	410	143
Total	320	103	56	51	44	6	23	410	89

period, 35 were related to the hosts of the parasites and 15 were unrelated. Twenty-two or 63 per cent of the related rats developed multiple peritoneal sarcomata in 10 to 787 days after injection. None of the unrelated rats of this group developed tumors. The average latent period was 286 days or more than 3 times the average for the experiments in Table I. In the first experiment shown in Table II, the 3 injected rats died 10 days after injection and 2 of those were found by microscopic examination to have early fibrosarcomata. In all other cases the tumors were probably the cause of death, since the rats at death showed gross involvement of the entire diaphragm, omentum, mesentery and the peritoneal surfaces of the abdominal wall and capsules of the liver, kidney, spleen and other organs. Typical examples are shown in Figs. 1 and 2. In the latter, the firm yellowish tumor tissue was arranged in thickened cords and sheets, giving the gross appearance of a malignant mesothelioma. Sections through the omental tumors in each case are shown in Figs. 3 and 4.

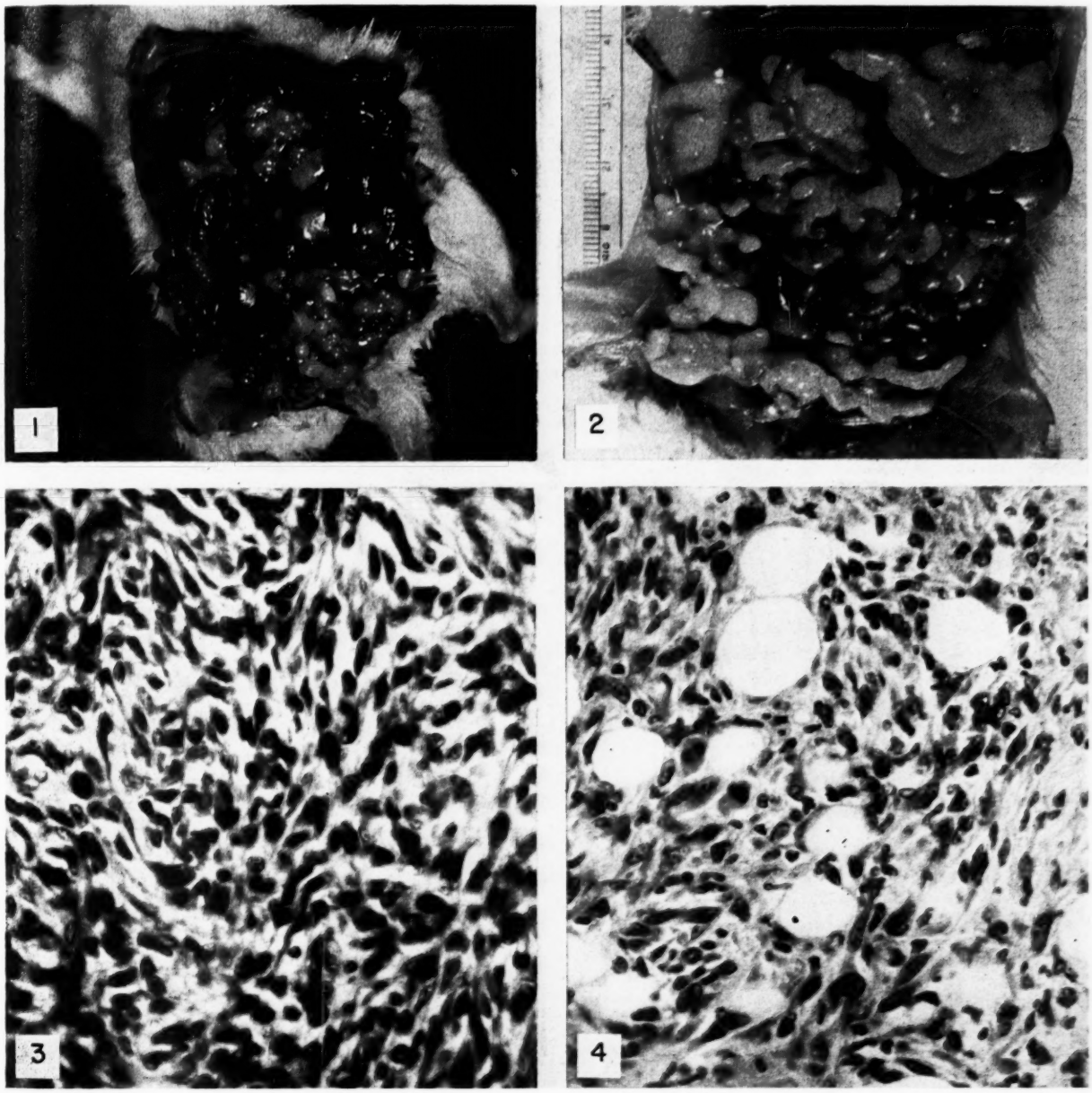

FIG. 1.—Rat with multiple peritoneal sarcoma, 62 days after injection of washed freshly ground *Taenia* larvae.
FIG. 2.—Rat with malignant mesothelioma, 410 days after injection of washed freshly ground *Taenia* larvae.
FIG. 3.—Section through omental sarcoma shown in Fig. 1. Mag. × 500.
FIG. 4.—Section through omental tumor shown in Fig. 2. Mag. × 500.

The procedure was varied in the different experi-

ments in order to further elucidate the conditions under which the active agent could be demonstrated and this in part accounts for some of the variation in latent period. For example, in the experiment under Feb. 1, 1940 in Table I, 6 rats were injected with whole larva suspension, while 6 other rats received the supernatant fluid after the suspension had been centrifuged at a moderate speed for 10 minutes; 4 rats received the sediment which was washed once by suspending it in saline, centrifuging at low speed, and then resuspended in saline. The latter sediment was found to consist chiefly of the characteristic calcium carbonate corpuscles which are normally present in the coelomic fluid of the larvae. In each group half of the rats were related to the hosts of the parasites and the others were unrelated. The 3 related rats which received the whole larvae suspension developed multiple peritoneal tumors in an average of 89 days, *i. e.,* 63, 95, and 110 days, respectively. One of the unrelated rats in this group developed multiple peritoneal tumors in 239 days. The 2 related rats which received the supernatant fluid developed tumors in 112 and 148 days, respectively, and the 2 related rats which received the centrifuged sediment, chiefly calcium carbonate corpuscles, developed tumors in 37 and 56 days, respectively. In the experiment under date of July 25, 1940 (Table II), the larvae were suspended in 0.3N acetic acid in an attempt to neutralize the natural alkalinity of the ground larvae, and in most instances this procedure prolonged the latent period or destroyed the activity completely. Solution of the calcium carbonate corpuscles in acetic acid or prolonged washing of the fresh larva sediment in saline destroyed the activity completely. In a few experiments all of the injected rats died within 24 hours after the injection. The high pH of the injected material was thought to be the cause of death of these rats. All experiments with desiccated, fractionated and filtered larvae have been negative and larvae frozen at −10° C. and stored at 4° C. for 2, 3, and 4 months have also proved negative.

Table II: The Number of Related and Unrelated Rats in Each Experiment Which Developed Multiple Peritoneal Sarcomata Following the Intraperitoneal Injection of a Saline Suspension of Washed, Ground *Taenia* Larvae Obtained from Rats with No Gross Tumor, and the Minimum, Maximum, and Average Latent Periods.

Date	Number of larvae	Total rats injected	Related rats: Over minimum latent period	Related rats: Number positive	Unrelated rats: Over minimum latent period	Unrelated rats: Number positive	Latent period in days: Minimum	Latent period in days: Maximum	Latent period in days: Average
Mar. 8, 1940	31	3	3	2	0	0	10	10	10
July 17, 1940	28	6	4	4	0	0	379	787	633
July 19, 1940	38	5	4	1	0	0	249		
July 24, 1940	18	4	4	1	0	0	617		
July 25, 1940	20	4	2	1	0	0	702		
July 25, 1940	54	6	4	2	0	0	410	459	435
Apr. 22, 1941	76	15	6	5	8	0	119	223	178
Apr. 29. 1941	94	16	8	6	7	0	60	76	69
Total	359	59	35	22	15	0	10	787	286

3. Rats of the same inbred line as the host from which the parasites are obtained respond more quickly and more frequently than unrelated rats.

4. The active agent appears to be associated with the calcium carbonate corpuscles of the parasite.

SUMMARY AND CONCLUSIONS

1. There appears to be an active agent present in washed, ground *Taenia* larvae which is capable of initiating multiple peritoneal sarcomata when injected into the peritoneal cavities of rats.

2. The agent is either more effective or else more abundant in parasites obtained from uninvolved cysts of hosts bearing induced *Cysticercus* sarcomas.

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Observations on the Carcinogenicity of 1,2,3,4-Dibenzophenanthrene and Its 9-Methyl and 10-Methyl Derivatives*

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In 1940 Badger *et al.* (1) reported that 1,2,3,4-dibenzophenanthrene was carcinogenic for mice when applied by painting the skin twice weekly with a 0.3 per cent solution in benzene, or by subcutaneous injection of 2.5 to 5 mgm. doses in 0.2 cc. of sesame oil, with repetition of the injection at intervals of 3 to 5 weeks according to the rate of disappearance of the quantity given previously. Of 20 mice painted with the compound, 5 developed cutaneous papillomata within a period of 212 to 258 days, and 8 developed cutaneous carcinomas within the same period. Of 30 mice that received the compound subcutaneously (the number of injections and the total dose administered were not stated), 5 developed sarcomas within a period of 144 to 185 days, and one other developed a spindle celled tumor of doubtful malignancy. The authors did not report upon methyl derivatives of this compound.

METHODS

In our experiments, suspensions of 1,2,3,4-dibenzophenanthrene and of its 9-methyl and 10-methyl derivatives were injected subcutaneously into young mice of the New Buffalo strain. This strain has been maintained by inbreeding in the Research Laboratories of Eli Lilly and Company since 1940, and was obtained from Dr. William S. Murray, of the New York State Institute for the Study of Malignant Diseases. A ratio of 100 mgm. of hydrocarbon to 1 cc. of menstruum was used. Tricapryllin was first employed as the suspending agent, but when our supply was exhausted, the ethyl ester of sesame oil was substituted. No difference in results attributable to this substitution could be detected. No mouse received a second injection.

The usual dose of 1,2,3,4-dibenzophenanthrene and of its 10-methyl derivative was 5 mgm. However, 8 mice received 10 mgm. and 2 received 15 mgm. of the parent compound; and 1 mouse received 15 mgm., 1 received 40 mgm., and 2 received 50 mgm. of the derivative. As anyone who has tried to inject such a suspension knows, these figures are not absolutely accurate, but they are close approximations. The dose of the 9-methyl derivative varied widely; 850 mgm. were injected into 32 mice, and some received very large doses. The structural formulas of these compounds are shown in Fig. 1.

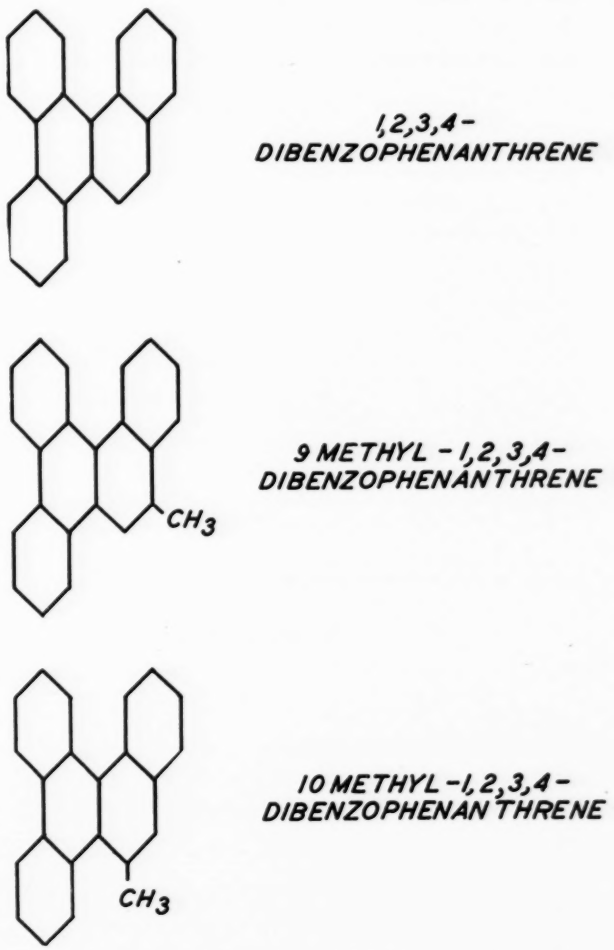

FIG. 1.—Structural formulas of 1,2,3,4-dibenzophenanthrene and its 9-methyl and 10-methyl derivatives.

* Presented at the 37th Annual Meeting of the American Association for Cancer Research at Atlantic City, New Jersey, March 11, 1946.

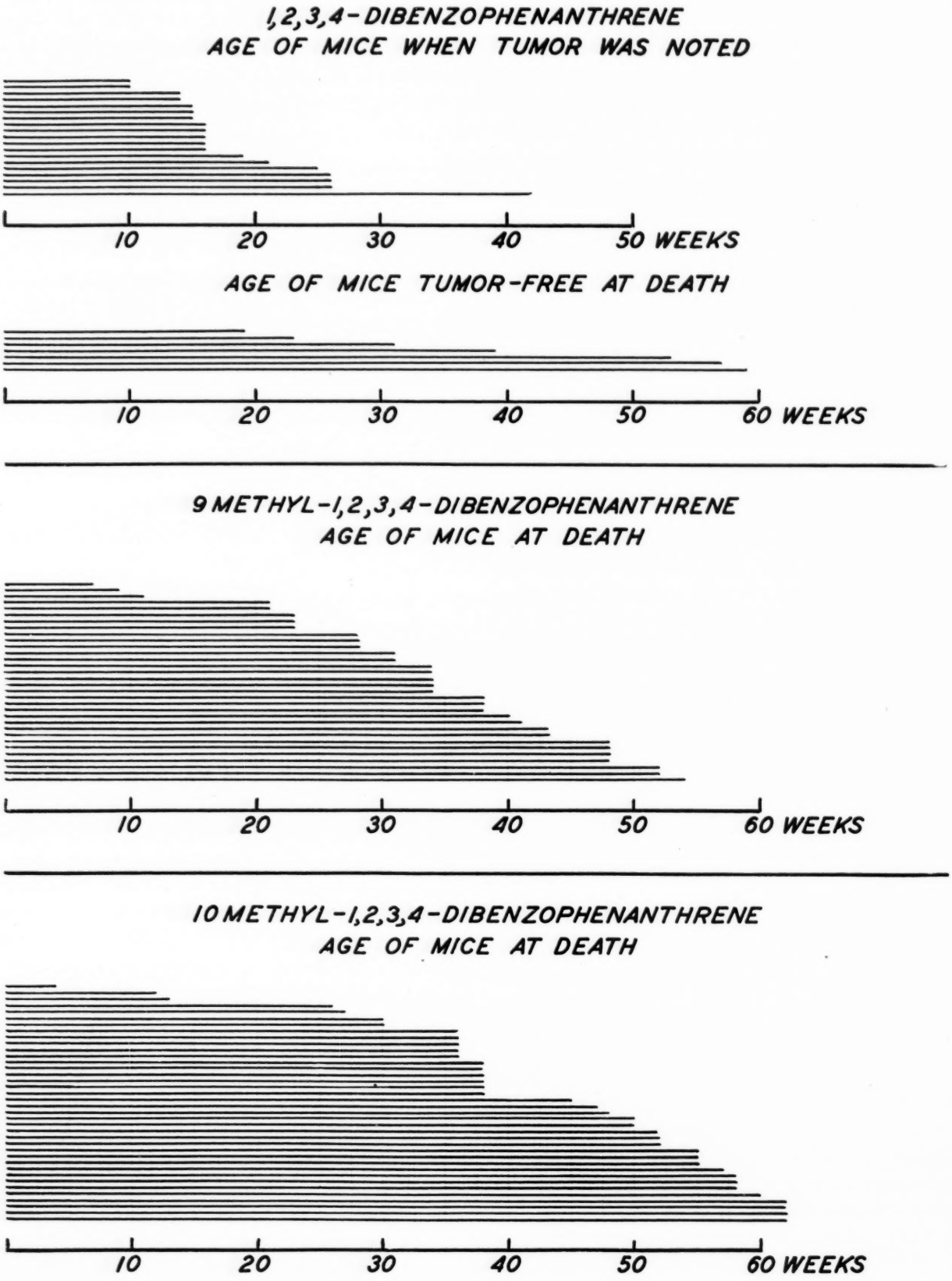

FIG. 2.—Graphical record of results of injections of 1,2,3,4-dibenzophenanthrene and its 9-methyl and 10-methyl derivatives. Each horizontal line represents 1 mouse.

RESULTS

Twenty-six mice were injected with 1,2,3,4-dibenzophenanthrene, and 19 ultimately developed tumors. One animal developed epidermoid carcinoma at the site of injection after a latent period of 26 weeks, and 3 developed both epidermoid carcinoma and sarcoma at the site of injection after latent periods of 25, 26, and 42 weeks. The other 15 mice developed sarcomas after latent periods of 10, 14, 15, 16, 19, 21, and 26 weeks. This is shown in Fig. 2, in which each horizontal line represents 1 mouse. The sarcomas, in general, were uniform in structure and were composed of fusiform cells arranged in bundles and whorls. Giant cells were relatively uncommon in most tumors, but were abundant in a few. Mitoses were usually numerous, and invasion of the abdominal muscle and of the panniculus carnosus was common. All these tumors appeared to be of fibroblastic origin. Fourteen mice developed ulcers at the site of inoculation, and in 10 of these, tumors appeared later. The 4 that developed carcinomas were in this group. The mice were not killed immediately upon the discovery of tumors, but were kept for about three weeks in order to obtain tumors of larger size.

Seven mice injected with hydrocarbon failed to develop tumors and lived from 19 to 59 weeks after inoculation (see Fig. 2). Four of these soon developed ulcers at the site of inoculation and possibly thereby lost enough hydrocarbon to render the dose ineffective. These mice died at 19, 23, 31, and 59 weeks after injection.

No tumors developed in mice that received the two methyl derivatives of 1,2,3,4-dibenzophenanthrene, and ulceration did not develop at the site of inoculation of either compound. Neither did tumors develop in control mice injected with tricapryllin or the ethyl ester of sesame oil. Thirty-two mice were inoculated with the 9-methyl derivative. Their survival ranged from 7 to 54 weeks and averaged 34 weeks. Thirty-eight mice received the 10-methyl derivative. Their survival ranged from 4 to 62 weeks and averaged 43 weeks (see Fig. 2).

DISCUSSION

Although the phenomenon is well known, it is nevertheless of considerable interest that addition of a methyl group to the 9 or 10 carbon atom of 1,2,3,4-dibenzophenanthrene changes the compound from a highly carcinogenic one to an apparently innocuous one. The fact that in our experiment tumors appeared earlier and in much larger percentage than in the study of Badger *et al.* (1) is probably due to our use of a suitable inbred strain instead of market mice.

SUMMARY

1. 1,2,3,4-Dibenzophenanthrene was found to be a highly potent carcinogen when injected subcutaneously into mice.

2. The 9-methyl and 10-methyl derivatives of this hydrocarbon failed to produce tumors when injected subcutaneously into mice.

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The Inhibition of the Carcinogenicity of *p*-Dimethylaminoazobenzene by Certain Detergents and the Effect of Diet on the Levels of Azo Dyes in Rat Tissues*

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It is well established that the rate at which liver tumors occur in rats fed p-dimethylaminoazobenzene can be altered greatly by dietary means. This subject has been reviewed through 1944 (15) and extended in more recent work (3-9, 16, 17). The dietary inhibitors and accelerators that have been reported include members of the vitamin B complex, certain lipids, some sulfur-containing amino acids, at least one mineral element, and various crude natural materials.

A consideration of the work of Frazer (1, 2) on the partition of olive oil between the lymphatic and portal systems of the intestine, particularly in the presence of a detergent, led us to try the effects of feeding low levels of two commercial synthetic detergents on the production of liver tumors in the rat. These substances were found to represent a new type of dietary inhibitor for *p*-dimethylaminoazobenzene and hence form the main subject of the present communication. Analyses of the content of basic azo dyes in the blood and livers of rats fed this dye in diets which affect its activity are also presented.

METHODS

Young adult male albino rats of the Sprague-Dawley strain, 140 to 190 gm. in weight, were kept in groups of 7 or 8 in screen-bottomed cages and fed the rations *ad libitum*. In the studies on carcinogenicity the dye was fed for 4 months and the livers examined by laparotomy at the end of this period. The animals were then continued on the same diets without the dye for another 2 months and a final examination of the livers was made at 6 months.

The control diet consisted of 12 parts of water-extracted casein (12), 4 parts of salt mixture, 5 parts of corn oil (Mazola), and 79 parts of glucose monohydrate (Cerelose) plus the following amounts of crystalline vitamins per kgm. added in cerelose mixtures: thiamine chloride, 3 mgm.; riboflavin, 2 mgm.; pyridoxine hydrochloride, 2.5 mgm.; calcium pantothenate, 7 mgm.; and choline chloride, 30 mgm. p-Dimethylaminoazobenzene was dissolved with heat in the corn oil and added at the level of 0.6 gm. per kgm. of diet. Each rat also received one drop of halibut liver oil by dropper once each month.

The detergents were obtained from the Carbide and Carbon Chemicals Corp., New York, and added to the control diet at the expense of the cerelose at a level of 0.25 per cent on a dry weight basis. Tergitol Penetrant No. 4 consisted of a 50 per cent aqueous solution of sodium 7-ethyl-2-methyl-undecanol-4-sulfate and Tergitol Penetrant No. 7 contained a 25 per cent aqueous solution of sodium 3,9-diethyl-tridecanol-6-sulfate plus small amounts of inorganic salts. These substances were fed throughout the 6 month periods in two separate series. A third experiment was conducted in which the dye was dissolved in mineral oil (light petrolatum, U.S.P. X) instead of corn oil. The rats fed mineral oil lost weight and appeared to be in poor health near the end of the period of dye-feeding. As a result the mineral oil was removed from the diet after 15 weeks for a 2 week period. After this the mineral oil was again fed but the rats were given in addition 2 drops of corn oil per week by dropper.

The analytical studies were performed on animals fed the diets described above as well as certain diets discussed in our previous papers (7, 15). These diets were fed for periods ranging from 2 to 17 weeks. At the end of these times the livers and bloods of the rats were analyzed for basic azo dyes (14) by a slight modification of the original method (11). Four cc. each of water, alcohol, and 11 N KOH were employed in the digestion of the tissues. A Cenco-Sheard Spectrophotelometer with an adapter for matched 13×100 mm. Pyrex culture tubes was used in measuring the

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final acid solutions of the dyes. *p*-Aminoazobenzene in the blood was determined by taking the solvent extracts to dryness *in vacuo* and then transferring the residues to the colorimeter tubes in a few cc. of the same pure solvent. Three cc. of 7 N HCl were then added and the tube shaken well after capping with a well-washed rubber stopper. A light centrifuging was needed to clarify the acid layer for the colorimetric measurement. When the 3 dyes were determined in liver each dye was eluted by suction from the chromatograph column into a colorimeter tube and the acid extractions carried out as above. In many of these analyses the livers were perfused *in situ* with 100 cc. of 2 per cent sodium citrate in order to remove the *p*-aminoazobenzene contained in the red blood cells (14).

either Penetrant No. 7 or Penetrant No. 4. No toxic symptoms were noted in the rats fed the detergents.

An attempt was made to duplicate the poor absorption of fat and dye that may possibly occur in rats fed detergents by feeding the dye in mineral oil instead of corn oil. The third series in Table I illustrates the strong protective effect of this diet. The incidences of tumors in the rats fed the corn oil were those usually observed but no tumors occurred in the mineral oil group by 4 months or by 6 months in the 8 survivors. These animals ate more food than the controls although probably less was absorbed since they lost weight during the dye feeding period. An attempt was made to improve their nutrition by removing the mineral oil at the time the dye was removed from the diet but 6 animals died shortly after laparotomy.

TABLE I: THE EFFECT OF CERTAIN DETERGENTS AND MINERAL OIL ON THE CARCINOGENICITY OF *p*-DIMETHYLAMINOAZOBENZENE

Diet	Average weight at			Average food consumption, gm./rat/day	Survival* at 4 mos.	Hepatomas† at		Negative survivors at 6 mos.	Gross cirrhosis at 4 months		
	Start, gm.	4 mos., gm.	6 mos., gm.			4 mos.	6 mos.		None	Moderate	Severe
Control (5% corn oil)	161	213	—	9.7	15/15	11/15	14/15	1	1	8	6
Control (5% corn oil) +0.25% Penetrant No. 7	157	240	258	10.3	15/15	0/15	0/15	15	13	2	0
Control (5% corn oil)	168	234	265	9.5	16/16	4/16	10/16	6	5	9	2
Control (5% corn oil) +0.25% Penetrant No. 7	168	244	261	10.2	14/15	0/14	0/14	13	12	2	0
Control (5% corn oil) +0.25% Penetrant No. 4	169	245	267	10.0	15/15	0/15	0/15	15	12	3	0
Control (5% corn oil)	217	229	310	9.8	15/15	6/15	12/15	3	7	5	3
5% Mineral oil	212	178	198	12.8	14/15	0/14**	0/14	8	14	0	0

* Survival = Number living over number at start.
† Hepatomas = Number of hepatomas over number surviving at 4 months.
** = A dermatitis indicative of a deficiency of fatty acid was observed between 3–4 months.

RESULTS

The effect of feeding the Tergitol Penetrants Nos. 4 and 7 on the carcinogenicity of *p*-dimethylaminoazobenzene is summarized in Table I. All of the rats fed the detergents consumed somewhat more food than the controls and they also gained more weight. Only two animals in the 3 series of experiments died before 4 months. While the incidence of tumors in the rats fed the control diet in the first series was unusually high for this diet, viz., 80 per cent and 93 per cent at 4 and 6 months respectively, this only serves to emphasize the protective effect offered by the inclusion of 0.25 per cent of Penetrant No. 7 in the diet. No tumors were noted by 6 months in animals fed this diet and the livers were nearly free of gross cirrhotic lesions. The strong protective effect of this detergent was confirmed in the second series. While the control group exhibited quite normal tumor incidences of 25 per cent and 63 per cent at 4 and 6 months respectively, no tumors were noted by 6 months in the rats fed the dye in the same diet plus 0.25 per cent of

At 4 months all of the rats fed mineral oil exhibited scaly paws and tail characteristic of a deficiency of linoleic acid. This syndrome was not noted in the rats fed the detergents.

Although it seemed possible that the protective effects of feeding these detergents and mineral oil might be due to poor absorption of the dye two types of observations did not support such a conclusion. Qualitative examinations of the feces by crushing them in 7 N HCl failed to reveal any of the pink colors given by the aminoazo dyes in this reagent; this test can reveal the presence of as little as 20 μgm. of dye. At present the only feasible way of studying the absorption and transport of the azo dye is to analyze for the three basic azo dyes found in the liver (14) and for *p*-aminoazobenzene in the blood. Extensive analyses to be reported in another paper (10) have shown that in any given rat the concentration of *p*-aminoazobenzene in the blood parallels the concentration of *p*-dimethylaminoazobenzene in the diet. A few data illustrating this point are given in Table II. When

the level of dye in the diet was varied between 0.01 per cent and 0.06 per cent the amount of *p*-aminoazobenzene in the blood was found to be roughly in proportion. The same conclusion can be drawn as to the levels of the three basic azo dyes found in the liver although here greater variations in concentration occurred. Table II also contains the results of analyses performed on several series of rats fed the various diets which are known to affect the carcinogenic activity of *p*-dimethylaminoazobenzene. Considerable variation was encountered from series to series in the level of *p*-aminoazobenzene in the bloods of rats fed 0.06 per cent of the dimethyl dye in the control diet. This variation was also evident in the previous data on this point (8, 14) and its cause is unknown. However, in the present analyses the levels of this dye in the blood

TABLE II: THE EFFECT OF DIET ON THE LEVELS OF BASIC AZO DYES IN THE LIVERS AND BLOOD OF RATS FED p-DIMETHYLAMINOAZOBENZENE

(Figures given as averages and ranges)

Diet	No. of rats	Time diet was fed (wks.)	μgm. dye/gm. fresh liver DAB*	MAB*	AB*	μmg. AB / cc. whole blood	Comment
Control (0.06% dye)	2	3	.24 (0.22–0.25)	.09 (0.08–0.09)	.49 (0.43–0.54)	10.8 (9.6–12.2)	*Ad libitum* feeding, livers perfused
Control (0.03% dye)	2	3	.13 (0.09–0.15)	.05 (0.04–0.06)	.13 (0.07–0.19)	3.9 (3.5–4.3)	"
Control (0.01% dye)	2	3	.08 (0.04–0.11)	.07 (0.02–0.06)	.12 (0.09–0.15)	0.86 (0.73–1.0)	"
Control (5% corn oil)	3	3	.47 (0.36–0.65)	.23 (0.17–0.30)	1.0 (0.76–1.4)	18.2 (17.2–19.4)	"
Control (5% corn oil) +0.25% Detergent 7	3	3	.36 (0.17–0.56)	.20 (0.11–0.32)	0.92 (0.48–1.6)	15.1 (13.8–15.8)	"
Control (5% corn oil) +0.25% Detergent 4	3	3	.31 (0.25–0.39)	.14 (0.09–0.19)	.65 (0.37–0.87)	15.6 (14.1–16.8)	"
Control (5% corn oil)	3	2	.67 (0.52–0.79)	.29 (0.22–0.33)	1.9 (1.6–2.5)	11.0 (10.4–11.3)	"
20% Corn oil	3	2	.48 (0.43–0.58)	.14 (0.13–0.16)	.79 (0.55–1.0)	11.1 (9.9–12.5)	"
Low fat	3	2	.47 (0.28–0.61)	.22 (0.12–0.34)	1.3 (0.81–2.0)	11.2 (8.1–16.0)	"
5% Mineral oil	3	2	.46 (0.43–0.50)	.21 (0.20–0.24)	1.6 (1.4–2.0)	9.0 (7.3–11.7)	"
Control (5% corn oil)	4	2	.34 (0.14–0.51)	.16 (0.06–0.22)	.66 (0.36–1.1)	24.0 (17.9–29.7)	Food intake = 11 gm./rat/day. Livers perfused
5% HCNO**	4	2	.29 (0.23–0.38)	.17 (0.13–0.21)	.62 (0.38–0.73)	18.2 (17.1–20.1)	"
Vitab-Crude Casein	4	2	.42 (0.20–0.76)	.24 (0.16–0.39)	1.0 (0.63–1.7)	24.2 (22.3–27.3)	"
Vitab-Crude Casein + High Riboflavin†	4	2	.19 (0.08–0.26)	.15 (0.08–0.20)	.43 (0.19–0.76)	24.5 (17.7–29.4)	"
Control (5% corn oil)	4	7	.50 (0.40–0.57)	.26 (0.22–0.29)	2.3 (1.9–2.9)	17.5 (16.5–19.1)	Food intake = 10 gm./rat/day. Livers not perfused
	4	13	.37 (0.30–0.42)	.22 (0.20–0.26)	1.2 (1.0–1.4)	12.2 (10.5–13.6)	"
	4	17	.26 (0.18–0.37)	.16 (0.08–0.27)	1.6 (1.0–2.4)	13.0 (11.6–15.2)	"
5% HCNO	4	7	.31 (0.20–0.55)	.19 (0.10–0.38)	2.0 (0.3–3.3)	10.2 (7.7–12.8)	"
	4	13	.41 (0.30–0.60)	.22 (0.15–0.38)	0.9 (0.4–1.3)	11.5 (8.6–13.6)	"
	4	17	.29 (0.22–0.32)	.16 (0.08–0.22)	1.1 (0.6–1.6)	6.3 (2.9–8.1)	"
Control + High Riboflavin†	4	7	.36 (0.30–0.41)	.20 (0.18–0.23)	1.2 (1.0–1.4)	10.6 (8.8–12.9)	"
	4	13	.24 (0.19–0.29)	.11 (0.07–0.18)	1.1 (0.9–1.4)	10.1 (7.9–11.4)	"
	4	17	.38 (0.23–0.53)	.23 (0.14–0.32)	1.4 (1.0–1.8)	8.3 (7.7–9.0)	"

* DAB = *p*-Dimethylaminoazobenzene.
MAB = *p*-Monomethylaminoazobenzene.
AB = *p*-Aminoazobenzene.
** HCNO = Hydrogenated coconut oil.
† 10 mgm. riboflavin per kilo of diet.

were reasonably constant in rats fed a given diet in any given series. The data show that the inclusion of detergents in the control diet or the substitution of mineral oil for corn oil in this diet cause a small drop in the levels of dye in the blood and liver. Similar decreases were also obtained with other strongly protective diets such as those containing hydrogenated coconut oil or high levels of riboflavin or with a partially protective diet such as the low fat diet. Diets containing Vitab (a rice bran concentrate) or high levels of corn oil which accelerate the formation of hepatic tumors had an inconsistent effect on the levels of dyes in the blood and liver; slight changes in either direction were observed. It is of interest also that a gradual decrease in the levels of dyes in these tissues occurred in rats as the time of feeding *p*-dimethylaminoazobenzene increased. However, most of the changes noted are slight and based on averages of figures whose ranges are admittedly large and overlapping.

DISCUSSION

These experiments demonstrate that the inclusion of a low level of either of two synthetic detergents in an otherwise carcinogenic diet has a pronounced protective effect. The effect was obtained twice with one of these agents and a single trial with a similar compound yielded the same result. Complete protection was also obtained when the corn oil in the control diet was replaced by mineral oil although the general poor condition and survival of the rats in this experiment indicate that it should be repeated. These compounds possess general properties which at first sight seem likely to account for the protective action observed. For example, both the detergents and mineral oil might be expected to diminish the absorbability of fat soluble compounds such as the azo dye from the intestinal tract. However, the analytical data only partially substantiate such a suggestion. Qualitative examinations of the feces of rats fed the dye with those inhibitors failed to reveal any significant quantity of aminoazo dyes and although the average levels of the dyes in the liver and blood were somewhat lower in animals that received protective factors the variability and overlapping of figures make it difficult to draw any final conclusion. A greater change was produced in the levels of the dyes in the liver and blood by lowering the concentration of the dye in the diet from 0.06 to 0.03 per cent than occurs when any of the inhibitors were fed with 0.06 per cent of the carcinogen. Since 0.03 per cent of the dye in the synthetic diet used above did not produce any liver tumors by 6 months (13) it would be necessary to have information on the incidence of liver tumors and the amount of dyes in the tissues on intermediate levels of dye to decide whether the slightly lower average results that are produced by dietary inhibitors are significant. Our tentative conclusion is that the diets used in these experiments do not greatly change the absorption of the dye from the tract since they do not appear to alter to any large extent the levels of the parent dye and its metabolites in the liver and blood. Certainly further data are needed on the metabolic fate of the dye during the ingestion of inhibitors or accelerators.

The continued discovery of dietary conditions which alter the carcinogenicity of *p*-dimethylaminoazobenzene often has raised the question of the fundamental importance of these effects. Some of the factors known to alter the potency of the azo dyes may also have a similar effect on carcinogenesis by other agents, but this has yet to be shown conclusively. For this reason it is quite possible that several of the dietary agents that affect the azo dyes operate prior to the carcinogenic process and some of the inhibitors may simply hasten the destruction of the dye before it has an opportunity to initiate tumors. Furthermore, the wide variety of these dietary agents suggests that either the action of the azo dye is easily altered at many stages or that many of these agents act through a common factor. However, the existence of such a variety of dietary means also increases the chance that some of them may interfere with or participate in the fundamental steps by which the azo dye acts as a carcinogen. Thus much of the value of studies on diet and the carcinogenicity of *p*-dimethylaminoazobenzene probably consists in furnishing materials and techniques that can assist in the elucidation of the mechanism by which this dye initiates the formation of liver tumors. New types of dietary agents should be particularly useful in this respect.

SUMMARY

Seven groups of 15 rats each were fed 0.06 per cent of *p*-dimethylaminoazobenzene for 4 months in synthetic diets containing: 5 per cent of corn oil; or 5 per cent of corn oil plus 0.25 per cent of either of two commercial synthetic detergents, Tergitol Penetrants 4 and 7; or 5 per cent of mineral oil. The dye-free diets were then fed for 2 more months. In the 3 control groups fed the diet containing 5 per cent of corn oil the final incidences of liver tumors were 93, 63, and 80 per cent. No tumors occurred when the diets contained either detergent or when the corn oil was replaced by mineral oil. These substances represent new types of dietary inhibitors for *p*-dimethylaminoazobenzene in the rat.

Analyses were made for the three basic azo dyes in the liver and for *p*-aminoazobenzene in the blood of rats fed *p*-dimethylaminoazobenzene in various diets known to affect the activity of this dye. Neither pro-

tective nor stimulatory diets produced any large changes in the concentrations of the dyes in these tissues. These data provide no conclusive support for the possibility that the detergents, mineral oil, and other inhibitors interfere with the absorption or transport of the dye.

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The Effect of Certain Diets on Hepatic Tumor Formation Due to *m*-Methyl-*p*-Dimethylaminoazobenzene and *o'*-Methyl-*p*-Dimethylaminoazobenzene*

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It has repeatedly been demonstrated that the nature of the diet can influence the rate at which hepatic tumors develop in rats fed *p*-dimethylaminoazobenzene. Dietary factors that retard the formation of such tumors include riboflavin (6, 14), hydrogenated coconut oil (10, 11) and several others. Dietary factors that accelerate tumor formation under these conditions include the crude mixture of extractives in commercial concentrate of rice bran (13, 14). The evidence has been summarized in a recent review (17).

While the experiments themselves are reproducible, the significance of these dietary effects on carcinogenesis is still open to question. Thus it has been pointed out (13, 17) that tumors of most other organs, whether spontaneous or induced by agents such as carcinogenic hydrocarbons or ultraviolet light, are not nearly so sensitive to diet as liver tumors due to *p*-dimethylaminoazobenzene, and hence one may ask whether the dietary factors are concerned with the fundamental carcinogenic reaction, or whether they act primarily by destroying or preserving the azo dye used as a carcinogen.

One experimental approach to this question would be to determine the effect of diet on the induction of hepatic tumors by agents other than *p*-dimethylaminoazobenzene. For if the carcinogenic reaction were involved, a similar response to diet might be expected of all carcinogens for that organ. On the other hand, if diet merely alters the rate at which carcinogenic chemicals are destroyed in the body, different responses to diet could result when different carcinogens are used, although a similarity in response is also possible if the same chemical grouping as, for example, the azo linkage, is involved in the metabolic destruction of a group of compounds.

In a previous study (8) the development of tumors due to *p*-monomethylaminoazobenzene was found to be retarded by both riboflavin and hydrogenated coconut oil, although these dietary factors were somewhat less effective quantitatively against the monomethyl dye than against *p*-dimethylaminoazobenzene.

MATERIALS AND METHODS

The present study deals with two related carcinogens, *m'*-methyl-*p*-dimethylaminoazobenzene and *o'*-methyl-*p*-dimethylaminoazobenzene. The former is highly carcinogenic (9, 4); the latter only moderately so (9). Both dyes were fed in 4 basal rations known to affect the development of tumors due to *p*-dimethylaminoazobenzene.

Young adult Sprague-Dawley rats were used throughout. They were placed in groups of 7 or 8 in screen bottom cages, and were given food and water *ad libitum*. The experiments were performed in series, in each of which the animals were divided into 4 or 5 groups of 15 rats each. All groups within a series received the same concentration of azo dye in the ration and for the same length of time but each group received a different basal ration (Table I). The various series differed from one another in the concentration of the dye fed, and in the duration of the experiment: from 0.026 per cent to 0.048 per cent of m'-methyl-*p*-dimethylaminoazobenzene fed for 2½ to 7 months (Table II). *o'*-Methyl-*p*-dimethylaminoazobenzene was fed at 0.096 per cent of the diet for 4 months. This is 1.5 times the molar concentration of azo dye used in most of our previous dietary studies (17). In most of the series the livers were examined by laparotomy at the end of the dye feeding period. Thereafter the rats were fed the appropriate basal diet, free from the dye, for another 2 months when the final examination for tumors was made. At the lowest concentration of the *m'*-methyl dye fed (0.026 per

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cent) a few animals from each group were examined by laparotomy at 4 months and again at 5½ months, but since no evidence of cirrhosis or liver tumors was apparent, the entire group was continued on the dye throughout the experiment. By 7 months large tumors were palpable in a few of the animals and the experiment was discontinued at that time.

The diets selected were those which had proved most effective in altering the rate at which hepatic tumors form when the carcinogen is 0.06 per cent of *p*-dimethylaminoazobenzene fed for 4 months. Diet I is the synthetic ration used as a control in most of our previous studies with *p*-dimethylaminoazobenzene (14, 17). At a level of 0.06 per cent for 4 months the incidence of tumors at 6 months ranged between 53 and 80 per cent, and in any series the incidence of hepatomas could be either increased or decreased from the basal figure depending upon appropriate changes in the diet. Diet II, containing the rice bran concentrate "Vitab," has consistently been observed to increase the rate of tumor formation (11, 13, 17). The substitution of hydrogenated coconut oil for corn oil delays the development of hepatic tumors due to *p*-dimethylaminoazobenzene (10, 11). Diet IV contained 10 mgm. of riboflavin per kgm. of ration. This amount of the vitamin is sufficient to retard tumor formation when the dye is *p*-dimethylaminoazobenzene (14), although it is relatively ineffective when the dye is *p*-monomethylaminoazobenzene (8). Accordingly, in one series the level of riboflavin was increased to 20 mgm. per kgm. of ration, Diet V. At this level riboflavin effectively inhibits the formation of neoplasms due to *p*-monomethylaminoazobenzene (8).

Table I: Composition of Diets

	I	II	III	IV	V
	Synth. control	Rice bran [1]	HCNO [2]	Riboflavin	Extra high riboflavin
	Gm. per kgm.				
Cerelose [3]	790	770	790	790	790
Casein (crude)	—	120	—	—	—
Casein (alc. extr.) [4]	120	—	120	120	120
Wesson salts	40	40	40	40	40
Corn oil	50	50	—	50	50
Hydrog. coconut oil	—	—	50	—	—
Vitab [1]	—	20	—	—	—
	Mgm. per kgm.				
Riboflavin	2.0	0.5	2.0	10.0	20.0
Thiamine HCl	3.0	—	3.0	3.0	3.0
Ca Pantothenate	7.0	—	7.0	7.0	7.0
Pyridoxine HCl	2.5	—	2.5	2.5	2.5
Choline chloride	30.0	—	30.0	30.0	30.0

[1] "Vitab Rice Bran Concentrate," obtained from National Oil Products Co., Harrison, New Jersey.
[2] HCNO = Hydrogenated coconut oil.
[3] Pure glucose monohydrate obtained from Corn Products Refining Company.
[4] For preparation see reference 14.

RESULTS

Tumors developed rapidly on all diets containing 0.032 per cent of *m'*-methyl-*p*-dimethylaminoazobenzene or more (Table II), and in general the rate at which they developed did not appear to be very greatly modified by diet. Thus, the rice bran diet II, which accelerates tumor formation due to *p*-dimethylaminoazobenzene, exerted only a small stimulatory effect in the present experiments with the *m'*-methyl dye. The greatest effect due to the rice bran concentrate, "Vitab," was in a series in which 0.048 per cent of the dye was fed for 2½ months. The final incidence of tumors 2 months later was 64 per cent on the control synthetic diet I and 87 per cent in the group receiving the rice bran concentrate (Table II). In a second series under the same conditions, the percentages were 40 and 54 per cent, respectively, while with all other amounts of the *m'*-methyl dye the effect of the rice bran extract was still less.

The hydrogenated coconut oil appeared to have no consistent effect on the development of hepatomas due to *m'*-methyl-*p*-dimethylaminoazobenzene. In 3 series the rats receiving the coconut oil had fewer tumors than those on corn oil, while in the other 3 series the animals fed the hydrogenated fat had as many or more neoplasms than their control groups (Table II). These results are in sharp contrast to our previous studies in which hydrogenated coconut oil proved to be a very effective inhibitor against the formation of

TABLE II: THE EFFECT OF VARIATION IN DIET ON THE CARCINOGENICITY OF *m'*-METHYL-*p*-DIMETHYLAMINOAZOBENZENE IN RATS

Per cent of dye in diet	Diet	Average starting weight, gm.	Average weight at end of dye feeding, gm.	Average food consumption, gm /rat/day	Time dye was fed, mo.	Gross cirrhosis at end of dye feeding: None, mild, no.	Gross cirrhosis at end of dye feeding: Mod. severe, no.	Survival at end of dye feeding period,* no.	Liver tumors**: End of dye feeding, no.	Liver tumors**: 2 months later: no.	Liver tumors**: 2 months later: %	Negative survivors 2 months later
0.048	Synth. (I)	178	181	10.6	3	3	10	13/15	6/13	11/13	83	2
0.048	HCNO (III)	168	180	10.6	3	6	8	14/15	6/14	12/14	86	2
0.048	Riboflavin (IV)	178	212	11.4	3	6	9	15/15	3/15	9/15	60	4
0.048	Synth. (I)	163	143	9.0	2.5	6	8	14/15	5/14	9/14	64	1
0.048	Rice bran (II)	165	173	8.8	2.5	7	8	15/15	5/15	13/15	87	1
0.048	HCNO (III)	157	193	9.8	2.5	6	6	12/15	2/12	6/12	50	3
0.048	Riboflavin (IV)	143	202	10.0	2.5	2	11	13/14	3/13	10/13	77	2
0.048	Synth. (I)	165	188	10.3	2.5	10	5	15/15	1/15	6/15	40	3
0.048	Rice bran (II)	163	170	10.7	2.5	7	6	13/15	0/13	7/13	54	5
0.048	HCNO (III)	162	179	10.1	2.5	11	3	14/15	0/14	6/14	43	6
0.048	Riboflavin (IV)	159	183	9.7	2.5	14	1	15/15	0/15	3/15	20	8
0.048	Extra high ribo. (V)	170	183	10.6	2.5	11	4	15/15	1/15	6/15	40	7
0.040	Synth. (I)	183	218	10.0	4	5	11	16/16	15/16	16/16	100	0
0.040	Rice bran (II)	188	242	9.7	4	6	8	14/16	7/14	13/14	93	1
0.040	HCNO (III)	188	230	10.6	4	5	10	15/16	13/15	14/15	93	1
0.040	Riboflavin (IV)	191	242	11.9	4	7	8	15/16	5/15	11/15	73	3
0.032	Synth. (I)	190	241	11.7	4	5	10	15/16	9/15	12/15	80	3
0.032	Rice bran (11)	189	244	11.0	4	6	7	13/15	4/13	12/13	92	0
0.032	HCNO (III)	189	243	12.4	4	10	5	15/15	5/15	8/15	53	5
0.032	Riboflavin (IV)	185	247	11.1	4	10	5	15/15	5/15	7/15	47	6
0.026	Synth. (I)	174	209	9.6	7	1	2	14/15	3/14	—	21†	—
0.026	Rice bran (II)	175	204	9.4	7	1	2	14/15	5/14	—	36†	—
0.026	HCNO (III)	171	200	9.7	7	0	2	11/15	4/11	—	36†	—
0.026	Riboflavin (IV)	174	222	9.8	7	1	2	14/15	1/14	—	7†	—

* Survival = number living over number at start.

** Liver tumors = number with liver tumors over number surviving at end of dye feeding period.

† At 7 months.

tumors due to a related carcinogen, *p*-dimethylaminoazobenzene (10, 11).

The only diet tested which inhibited the induction of hepatomas due to the *m'*-methyl dye was diet IV containing 10 mgm. of riboflavin per kgm. of diet. This diet lowered the incidence of tumors in 5 of the 6 series attempted. The percentages of tumors developing on the control diet I and the riboflavin diet IV under the various dosages of the *m'*-methyl dye are as follows:

Diet I Control	Diet IV Riboflavin
83	60
64	77
40	20
100	73
80	47
21	7

(see Table II for details). But while the trend suggests that riboflavin had reduced the carcinogenic effectiveness of *m'*-methyl-*p*-dimethylaminoazobenzene somewhat, it is also evident that the quantitative effect of the vitamin is not very great on tumor formation due to this carcinogen. Furthermore, in the single series in which riboflavin was fed at a still higher level, 20 mgm./kgm., diet V, there was no inhibition whatever.

The results of a single series with the weaker carcinogen *o'*-methyl-*p*-dimethylaminoazobenzene fed as 0.096 per cent of the diet for 4 months suggested that the carcinogenicity of this compound also, may be altered somewhat by diet. Nine of 14 rats on the control synthetic diet I, or 64 per cent, developed tumors by 6 months (Table III). When diet II containing the rice bran concentrate was fed, the tumor incidence was 73 per cent. The diets containing the hydrogenated coconut oil or extra riboflavin reduced tumor formation somewhat, the incidences being 46 and 38 per cent respectively. Each of the diets studied, therefore, modified the carcinogenicity of the *o'*-methyl-derivative qualitatively as that due to *p*-dimethylaminoazobenzene (17). Quantitatively, the effects of the various diets against the *o'*-methyl dye were greater than the average responses of these diets in the 6 series in which the *m'*-methyl dye was employed (Tables II and III); the diets were not as effective quantitatively against the *o'*-methyl dye as against *p*-dimethylaminoazobenzene.

DISCUSSION

The present experiments indicate that the carcinogenicity of *m'*-methyl-*p*-dimethylaminoazobenzene can be modified somewhat by diet, although the dietary agents studied were less effective than they are against *p*-dimethylaminoazobenzene. Thus, hydrogenated coconut oil has no consistent action while the rice bran concentrate caused only a slight stimulation. Riboflavin showed a somewhat greater tendency to counteract the effects of the *m'*-methyl dye than the other dietary factors studied, but even riboflavin was probably less than half as effective against *m'*-methyl-*p*-dimethylaminoazobenzene in the present study as its previously demonstrated potency against the effects of *p*-dimethylaminoazobenzene (17).

It is reccgnized that secondary factors which modify the rate of tumor formation are most evident when the dose of the carcinogen employed is borderline. For this reason too much emphasis should not be placed upon irregularities in tumor incidence noted when a relatively high concentration of the *m'*-methyl dye was fed for only 2½ months. Nevertheless, the relative insensitivity of the *m'*-methyl dye to diet did not appear to be due solely to the high carcinogenicity of the compound, for the effectiveness of the diets employed was not increased at a concentration of only 0.026 per cent of this azo dye in the diet (Table II). Under these conditions riboflavin still failed to furnish complete protection against the formation of tumors, even though only 21 per cent of the rats on the control diet had hepatomas when the experiment was terminated after 7 months of dye-feeding. Further evidence that resistance to diet does not necessarily parallel carcinogenicity is the observation that *o'*-methyl-*p*-dimethylaminoazobenzene is less sensitive to

TABLE III: THE EFFECT OF VARIATIONS IN DIET ON THE CARCINOGENICITY OF 0.096% *o'*-METHYL-*p*-DIMETHYLAMINOAZOBENZENE FED 4 MONTHS

Diet	Average starting weight, gm.	Average weight at 4 mo., gm.	Average food consum. gm./rat/day	Gross cirrhosis at 4 months		Survival at 4 mo.,* no.	Liver tumor at **			Negative survivors at 6 mo.
				None-mild, no.	Moderate-severe, no.		4 mo., no.	6 mo. No.	6 mo. %	
Synth. (I)	169	150	7.3	3	11	14/15	1/14	9/14	64	2
Rice bran (II)	172	157	7.7	6	9	15/15	2/15	11/15	73	3
HCNO (III)	176	185	9.3	10	3	13/14	4/13	6/13	46	4
Riboflavin (IV)	169	211	10.1	11	2	13/13	3/13	5/13	38	7

* Survival = number living over number at start.
** Liver tumors = number with liver tumors over number surviving at 4 months.

diet than *p*-dimethylaminoazobenzene, although the latter compound is the more active carcinogen (9) and (Table III).

Of the dietary factors studied riboflavin appears to have the most general action against hepatic tumors due to azo dyes, since some degree of protection has now been observed against 4 different carcinogens of this type: *p*-dimethylaminoazobenzene, *p*-monomethylaminoazobenzene, *o'*-methyl-*p*-dimethylaminoazobenzene and *m'*-methyl-*p*-dimethylaminoazobenzene, in that order. To the extent that riboflavin protects against all 4 compounds, it is possible that it is involved in the fundamental carcinogenic reaction, but the rather wide discrepancy in the degree of resistance conferred by the vitamin against *p*-dimethylaminoazobenzene as compared to the *m'*-methyl derivative suggests that not all of the resistance to the former compound is concerned with carcinogenesis.

Similar considerations applied to the results obtained with hydrogenated coconut oil suggest that this material has little to do with the reaction by which azo dyes cause tumor cells to arise in the liver, for hydrogenated coconut oil exerted no consistent protection against tumors due to the *m'*-methyl dye. Again the high degree of protection observed against tumors due to *p*-dimethylaminoazobenzene would have to be ascribed to an effect on the metabolism of the dye itself. In this connection studies on such carcinogenic agents as *o*-aminoazotoluene, CCl_4 (2), and selenium (16) would be of interest for all are reported to be carcinogenic for rats or mice, although they presumably undergo quite different pathways of metabolic degradation. Aminoazotoluene is sensitive to certain crude diets; liver tumors due to this agent develop more readily when the basal diet consists largely of rice (1) than when it is wheat (1, 3), rye (7), or millet (15). On the other hand acetoaminofluorene is a carcinogen the activity of which appears to be insensitive to diet (5).

The present studies on the carcinogenicity of the *m'*-methyl- and *o'*-methyl-*p*-dimethylaminoazobenzene furnish a good basis for estimating their activity relative to *p*-dimethylaminoazobenzene. In previous experiments 53 to 80 per cent of rats fed 0.06 per cent of *p*-dimethylaminoazobenzene in the synthetic control diet for 4 months developed liver tumors by 6 months (17), as compared to an incidence of 80 per cent at this time in animals fed 0.032 per cent of the *m'*-methyl derivative for 4 months in the present series (Table II). Hence, the latter compound appears to be about twice as active as the parent dye. Similarly, since 0.096 per cent of *o'*-methyl-*p*-dimethylaminoazobenzene under the same conditions caused 64 per cent of the rats to develop neoplasms, the latter compound would seem to be about two-thirds as active as *p*-dimethylaminoazobenzene. Such comparisons are of course dependent on the conditions of the experiment and the relative activities will probably differ somewhat as the level of dye, the period of feeding, or the diet is varied.

SUMMARY AND CONCLUSIONS

1. *m'*-Methyl-*p*-dimethylaminoazobenzene was fed to rats in 4 different rations known to affect the formation of tumors due to *p*-dimethylaminoazobenzene. The *m'*-methyl dye was fed at several concentrations and for different periods of time.

2. In general diet was relatively ineffective in altering the rate of tumor development due to *m'*-methyl-*p*-dimethylaminoazobenzene. Hydrogenated coconut oil did not exert a consistent protective effect, while rice bran extract stimulated tumor formation only slightly.

3. Riboflavin usually retarded the formation of tumors due to the *m'*-methyl dye, but the effect of the vitamin was less than that previously observed when *p*-dimethylaminoazobenzene was the carcinogen.

4. Rice bran concentrate stimulated tumor formation due to *o'*-methyl-*p*-dimethylaminoazobenzene whereas either hydrogenated coconut oil or riboflavin retarded it. The effects of diet against this carcinogen were intermediate between those observed against *p*-dimethylaminoazobenzene and *m'*-methyl-*p*-dimethylaminoazobenzene.

5. The variable effects of diet against the different azo dyes suggest that riboflavin may retard tumor formation by interfering with the essential carcinogenic reaction, but that the other diets studied more probably exert their effects upon the carcinogen.

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Intracellular Distribution of Enzymes

II. The Distribution of Succinic Dehydrogenase, Cytochrome Oxidase, Adenosinetriphosphatase, and Phosphorus Compounds in Normal Rat Liver and in Rat Hepatomas

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Interest in the particulate components of the cell has achieved considerable prominence during the last few years largely as the result of the development of technics for the isolation of these cellular constituents in quantities sufficient for chemical and biochemical analysis. Thus, Bensley and his coworkers (4, 5, 19, 21, 22) have described the isolation of mitochondria, particulate glycogen, and a submicroscopic component of guinea pig liver. Claude has been instrumental in developing well defined methods for the isolation of large granules, microsomes, and chromatin threads from normal and neoplastic tissues (8, 9, 10, 11, 12). He has subjected these particulate components to elementary chemical analysis and more recently has described some of the enzymatic properties of the large granules and of the microsomes (11). A number of enzymes important to cellular metabolism were associated with the large granules while other enzymes were not associated with any particulate material and were apparently present in the cell in soluble form. None of the enzymes studied was found to be associated with the microsomes. The enzymatic properties of the nucleus have been studied in the case of liver by Dounce (13) but these studies have not been extended to malignant tissues because of technical difficulties encountered in the isolation of nuclei from these tissues (14, 15).

The importance to cancer research of a study of the particulate components of the cell is perhaps best emphasized by a few illustrations. In the first place, the viruses which cause several tumors have dimensions and chemical properties similar to those of particulate components isolated from normal cells (7, 8, 28). Furthermore, a preliminary report has indicated that the antigen which is specific for the Brown-Pearce tumor is associated with the microsome or small particle fraction of this tissue (24). Finally, Graffi (17, 18) has shown that the mitochondria of a large variety of tissues will preferentially absorb carcinogenic hydrocarbons *in vitro* and has presented some evidence that these particulate components are involved in the absorption of carcinogens painted on the skin of mice.

The present report describes the results of an investigation of some of the enzymatic and chemical properties of normal rat liver and rat hepatoma cells which have been separated by centrifugation into a nuclear fraction, a large granule fraction,[1] and an unfractionated residue. Although objections have been raised to a comparison of normal liver and hepatoma on the basis that these tissues are greatly different in their degree of differentiation (11), such a comparison would nevertheless seem to be of considerable interest since the two tissues do represent the original and final stages in the carcinogenic process.

MATERIALS AND METHODS

Tissues.—The tissues were removed as rapidly as possible from animals killed by decapitation and were homogenized in 9 volumes of alkaline water (distilled water brought to pH 9.5 by the addition of 2.0 ml. of 0.1 N NaOH per liter (see 9, 10, 31)) in the apparatus of Potter and Elvehjem (26). The hepatoma samples were obtained through the courtesy of Drs. J. A. and E. C. Miller, and B. E. Kline. These hepatomas had been induced in rats by the oral ingestion of p-dimethylaminoazobenzene.

Tissue fractionation.—The homogenates were separated into a nuclear fraction, a large granule fraction,[1] and an unfractionated residue by a centrifugation procedure described previously (31). The unfractionated residue consists of a supernatant and washings which remain after removal of nuclei and large granules and

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[1] As Claude has pointed out (10) the large granule fraction of the liver contains secretory granules and mitochondria in unknown proportions. The large granule fraction of the rat hepatoma on the other hand may be deficient in secretory granules.

is the fraction from which microsomes and glycogen have been isolated by the use of greater centrifugal forces (9, 10, 21, 22). The time which elapsed during the centrifugation was 4 to 5 hours.

Cytological methods.—The whole homogenate and each of the tissue fractions were examined in the bright and in the dark field microscopes. Smears were also made and stained with aniline-acid fuchsin-methyl green (3).

Enzyme assays.—Succinic dehydrogenase, cytochrome oxidase, and adenosinetriphosphatase (ATP-ase) were measured as described previously (16, 32). The activities of the original homogenate and of each of the fractions were determined simultaneously immediately after the fractionation had been completed.

Analytical methods.—Desoxypentose nucleic acid (DNA), pentose nucleic acid (PNA), and acid soluble, lipid, and "protein" phosphorus were determined by methods reported previously (29). Nitrogen was determined on the "phosphoprotein" fraction by the method of LePage and Umbreit (23). Dry weight was determined by drying aliquots of the tissue suspensions to constant weight *in vacuo* over P_2O_5.

RESULTS

The results of the enzyme assays and of the analyses are presented in Table I. The results were expressed in terms of 100 mgm. of fresh tissue or its equivalent so that data on the fractions could be compared with the data on the whole homogenate for recovery calculations. If such a comparison is made, it is seen that the amounts of the materials recovered in the tissue fractions agree well with the amounts found in the whole homogenate.

Cytological observations.—Microscopic examination of the fractions showed that the nuclear fraction contained some whole cells and large granules in addition to large numbers of intact nuclei which had clumped together in large masses. The large granule fraction contained large numbers of particles of a fairly uniform size which stained red with Bensley's aniline-acid fuchsin-methyl green (3). Nuclei and whole cells were apparently absent from this fraction and also from the unfractionated residue although the latter did contain some large granules.

Enzyme assay results.—The major portions of the succinic dehydrogenase and cytochrome oxidase present in the original homogenate were found to be associated with the large granule fractions of both tissues. In confirmation of previous results, however, the activity of these enzymes in the hepatoma was considerably lower than in the normal liver (33).

The distribution of ATP-ase in the tissues was of considerable interest because the activity of this enzyme had been shown to be essentially the same in

TABLE I: DISTRIBUTION OF ENZYMES, PHOSPHORUS-CONTAINING COMPOUNDS, PROTEIN NITROGEN, AND DRY MATERIAL IN FRACTIONS OF RAT LIVER AND HEPATOMA HOMOGENATES

Average values are given, with ranges of values in parentheses. The data on liver were reported previously (31). The data on hepatoma include 4 analyses with the exceptions of cytochrome oxidase and ATP-ase in which only 2 analyses were made.

Tissue	Tissue fraction	Enzyme activities			Phosphorus distribution per 100 mgm. fresh tissue					"Protein" nitrogen per 100 mgm. fresh tissue, gamma	Dry material per 100 mgm. fresh tissue, mgm.
		Succinoxidase*	Cytochrome oxidase*	ATP-ase‡	DNA,** gamma	PNA,** gamma	Nucleic acid, gamma	Acid soluble, gamma	Lipid, gamma	"Protein," gamma	
Liver	Original homogenate	383	1012	865	22.6	65.2	95	125	155	40.2	1970	31.9
	Nuclear fraction	25.4	54.6	231	23.4	4.9	27.2	4.8	19.0	6.1	197	3.3
	Large granule fraction	289	748	416	—	11.4	16.0	4.9	42.1	11.4	434	5.7
	Unfractionated residue	45.5	147	257	—	47.8	61.5	115	95.0	19.7	1250	23.3
Hepatoma	Original homogenate	86.7 (78.3–95.3)	322	802	52.3 (43.5–56.0)	62.1 (58.4–70.1)	112 (97–124)	93 (83–105)	109 (84–127)	24.1 (18–28.7)	1093 (792–1330)	21.3 (20.6–21.9)
	Nuclear fraction	10.0 (7.3–12.1)	29.4	99.5	50.0 (42.1–55.0)	7.4 (6.6–10.6)	52.3 (48–51.4)	4.8 (2.7–7.9)	16.9 (10.4–21.2)	9.6 (4.6–12.8)	265 (213–366)	5.3 (4.9–5.5)
	Large granule fraction	48.5 (45–56.4)	203	98	—	6.6 (4.6–8.5)	8.6 (6.5–9.7)	1.5 (1.0–2.2)	16.2 (13–20.1)	3.0 (2.1–3.7)	94 (89–100)	1.7 (1.3–1.8)
	Unfractionated residue	20.4 (15.1–26.6)	101	594	—	50.1 (44.4–58.1)	56.6 (42.9–63.3)	87.3 (79–101)	77.2 (59.8–88.1)	14.6 (11.3–18.1)	706 (408–918)	13.8 (12.1–14.7)

* The activities are expressed as cu. mm. O_2 taken up per 10 min. by the equivalent of 100 mgm. of fresh tissue.
‡ The activities are expressed as micrograms phosphorus liberated in 15 min. by the equivalent of 100 mgm. of fresh tissue.
** Phosphorus calculated from pentose measurements.

both tissues (27). This observation was confirmed in the present study (Table I). The distribution of the enzyme in the two tissues was radically different, however. In the case of the normal liver, about 48 per cent of the activity was found to be associated with the large granule fraction and 30 per cent with the unfractionated residue while in the hepatoma 75 per cent of the ATP-ase activity was found to be associated with the unfractionated residue and only 12 per cent with the large granule fraction. It would be of considerable interest to extend the ATP-ase studies to include an investigation of the specificity of the enzyme towards various substrates, a study of the degree of hydrolysis of ATP in the various fractions, and further fractionation of the tissues.

Analytical results.—The analyses for DNA showed that all of this nucleic acid was present in the nuclear fractions of both normal liver and hepatoma. Thus excellent chemical evidence was provided in support of the cytological observations which indicated that nuclei were present only in the nuclear fraction and also in support of the hypothesis that DNA is found only in the nucleus of the cell. The data also confirm previous observations made in this laboratory (30) which showed that DNA was present in much higher concentrations in the hepatoma than in the normal liver.

PNA, acid soluble and lipid phosphorus, "protein" nitrogen, and dry material were found mainly in the unfractionated residue. In harmony with the idea that the large granules are complexes of protein and phospholipid (10), lipid phosphorus was found to be more concentrated per mgm. of dry material in the large granule fractions. It is also of interest to note that the nuclear fraction of the hepatoma contains more dry material and "protein" nitrogen than does the nuclear fraction of normal liver while in the case of the large granule fraction the situation in the two tissues is reversed.

DISCUSSION

The observation that the succinic dehydrogenase and the cytochrome oxidase activities of normal rat liver and of rat hepatoma were associated with the large granule fractions of these tissues introduces the possibility that the decreased activity of these enzymes observed in the hepatoma as compared to the normal liver (Table I), and (33), might be rationalized on the basis of a decreased amount of large granule material in the hepatoma cell. If this explanation were correct, the activity of these enzymes per mgm. of dry material and of protein nitrogen should be the same in the large granule fractions of the two tissues. Table II shows the activities of these enzymes per mgm. of dry material and of "protein" nitrogen. It is evident that the enzyme activities when expressed in these terms are more similar for the large granule fractions of the normal liver and the hepatoma than are the enzyme activities of the original tissues. Thus it would seem that the decreased activities of these enzymes in the hepatoma could be explained at least in part on the basis of a diminution in the large granule fraction of the hepatoma cell. Although the data on cytochrome oxidase are in accord with such an explanation, the data on succinic dehydrogenase show a considerable discrepancy between the activity of this enzyme per unit of protein or dry weight in the large granule fraction of the hepatoma as compared with the normal liver. In addition, it must be pointed out that the chemical composition of the hepatoma granules [1] is considerably different from the composition of the large granules of the liver; the former contain more lipid phosphorus and PNA and less "protein" nitrogen than do the latter. Thus, although the hepatoma cell does contain less large granule material than the normal liver cell, it is apparent that this difference does not provide a complete explanation for the decreased enzyme activities observed in the hepatoma. Never-

TABLE II: ENZYME ACTIVITIES PER MGM. DRY MATERIAL AND PER MGM. PROTEIN NITROGEN OF THE FRACTIONS OF NORMAL RAT LIVER AND HEPATOMA

		Activity per mgm. dry weight			Activity per mgm. "protein" nitrogen		
Tissue	Fraction	Succin-oxidase*	Cytochrome oxidase*	ATP-ase**	Succin-oxidase*	Cytochrome oxidase	ATP-ase**
Normal liver	Original homogenate	12.0	31.7	27.2	194	514	439
	Nuclear	7.7	16.6	70.0	129	278	1170
	Large granule	50.7	131	73.0	665	1720	960
	Unfractionated residue	2.0	6.3	11.1	36.4	118	206
Hepatoma	Original homogenate	4.1	15.1	37.6	79.5	295	735
	Nuclear	1.9	5.5	18.8	37.8	111	376
	Large granule	28.5	119	57.7	516	2160	1040
	Unfractionated residue	1.5	7.3	43.0	28.9	143	843

* Cu. mm. O_2 uptake per 10 min.
** Micrograms phosphorus liberated per 15 min.

theless, in the case of enzymes which are associated with large granules or other particulate material, variations in the amount of the particulate material in the cell must be considered as a possible explanation for variations in the enzyme activities which occur under different physiological conditions. Thus it would be of considerable interest with reference to the association of enzymes with granules to re-examine the variations of the succinoxidase activity observed in vitamin deficiencies (1, 2, 20), pregnancy (25), and after adrenalectomy and thyroid feeding (34).

The data on ATP-ase (Table II) show that the activity of this enzyme was much the same in the large granule fraction of the normal liver and of the hepatoma. In the case of the hepatoma, however, only a small portion of the original ATP-ase activity was associated with the large granule fraction while in the liver most of the ATP-ase activity was associated with this fraction (Table I). The largest portion of the ATP-ase present in the hepatoma was found to be associated with the unfractionated residue. Thus the findings on ATP-ase also suggest a loss of large granule material as a characteristic of the malignant change. In the case of this enzyme, however, the activity which was associated with the large granule fraction of the liver appeared in the unfractionated residue of the hepatoma cell in contrast to the enzymes of the succinoxidase system which were lost from the cell.

An important point and a difficult one to decide is whether the DNA content of hepatoma nuclei is greater than the DNA content of liver nuclei. A comparison of the ratio of DNA to dry material in the nuclear fractions of the two tissues indicates that the DNA content of hepatoma nuclei is greater (Table I). The same conclusion was reached in a previous report from this laboratory upon different evidence (30). On the other hand Dounce found that the DNA content of transplanted hepatoma 31 nuclei was about 50 per cent as great as the DNA content of liver nuclei (15). His results were complicated by the fact that liver nuclei prepared at pH 2.4 had apparently lost histone while nuclei prepared at pH 6.0 had lost some nucleic acid. A similar situation was inferred to exist in the case of the hepatoma nuclei although no evidence was advanced in support of this assumption. Our own results would seem to be independent of these difficulties since the nuclear fraction was prepared at a pH of about 7.0 and because all of the DNA present in the original tissue was recovered in the nuclear fraction. On the other hand our results are open to the criticism that the nuclear fractions are admittedly impure. If this factor is considered, it would seem that the differences between the nuclear fraction of the hepatoma and the nuclear fraction of the normal liver would be greater since the latter would be more likely to be contaminated with large amounts of large granule material than the former.

The data presented in Table I support the suggestion advanced previously on entirely different evidence that the hepatoma contains approximately twice as many cells per volume of tissue as does the normal liver (30). This follows from the data on the DNA and the dry material contents of the nuclear fractions of the two tissues if it is assumed that the DNA content per nucleus is approximately the same for the two tissues. If it is true that the hepatoma contains twice as many cells per volume of tissue as does normal liver, it is of interest to note that the amount of dry material found in the hepatoma cytoplasm (large granules plus unfractionated residue) is only about 50 per cent as great as the amount of dry material found in the liver cytoplasm. This is considerably less than would be predicted on the basis that the nucleus is the same size in both tissues (6) and occupies about 6 per cent of the volume of the liver cell (13).

From the data presented in this paper the following picture of the difference between the normal liver cell and the hepatoma cell can be obtained. The hepatoma tissue contains cells which are about one-half as large as the normal liver cells. The volume of the nucleus in both tissues is approximately the same, however, and therefore the volume of the cytoplasm of each hepatoma cell must be about one-half as large as the cytoplasm of the normal liver cell. The concentration of large granule material and of other cytoplasmic material is much less in the hepatoma cell than in the liver cell. The enzymatic properties of the cytoplasm have also been altered; a large portion of the activity of the succinoxidase system present in the normal liver cell is absent from the hepatoma cell while the distribution of ATP-ase in the hepatoma cell is different from that of the liver cell although the ATP-ase activities of the two tissues are essentially the same. The concentration and distribution of chemical compounds are also different in the cytoplasm of the hepatoma cell than in the normal liver cell. Thus it is evident that the differences between the normal and the malignant cell are exceedingly complex. A further understanding of these differences will undoubtedly be obtained when the studies are extended to include a larger number of enzymes, a more complete fractionation of the tissues, a study of embryonic and regenerating liver as well as livers obtained from animals at various stages of the carcinogenic process, and a wider variety of normal and cancerous tissues.

SUMMARY

1. Homogenates of normal rat liver and rat hepatomas were separated by centrifugation into a nuclear fraction, a large granule fraction, and an unfractionated residue.

2. Succinic dehydrogenase, cytochrome oxidase, adenosine triphosphatase, pentose and desoxypentose nucleic acids, acid soluble and lipid phosphorus, "protein" phosphorus and nitrogen, and dry material were determined on the original tissue homogenate and on each of the tissue fractions.

3. The activities of succinic dehydrogenase and cytochrome oxidase were much lower in the hepatoma than in the normal liver. However, the major part of the enzyme activities associated with the original tissues was found to be associated with the large granule fractions of these tissues, and the enzyme activities per unit of dry material or of "protein" nitrogen were similar in the large granule fraction of the two tissues. Thus the decreased enzyme activity observed in the hepatoma seemed to be due at least in part to a decrease in the amount of large granule material.

4. The activity of adenosinetriphosphatase was essentially the same in the hepatoma as in the normal liver although the distribution of the enzyme was profoundly different in the two tissues. In the latter about 50 per cent of the activity was associated with the large granules and 30 per cent with the unfractionated residue, while in the hepatoma 75 per cent of the enzyme activity was associated with the unfractionated residue and only 12 per cent with the large granules. The enzyme activity per unit of dry weight or of "protein" nitrogen was similar for both tissues in the large granule fraction.

5. The desoxypentose nucleic acid content of the hepatoma was found to be more than twice as great as that of normal liver. All of the desoxypentose nucleic acid present in the whole homogenates was recovered in the nuclear fractions. The increased content of this nucleic acid in the hepatoma was considered to be due to an increase in the number of cells in this tissue.

6. The pentose nucleic acid content of the two tissues was found to be essentially the same and the major part of this nucleic acid was found in the unfractionated residue. The nucleic acid content per mgm. of dry material was higher in the large granule fraction and in the unfractionated residue of the hepatoma than in normal liver.

7. The major portions of the other components measured were found in the unfractionated residue. The lipid phosphorus was more concentrated in the large granule fractions of the two tissues and more concentrated in the cytoplasm fractions of the hepatoma than in the corresponding fractions of normal liver.

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Phosphorus Compounds in Animal Tissues

IV. The Distribution of Nucleic Acids and Other Phosphorus-Containing Compounds in Normal and Malignant Tissues*

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Malignant tissues are distinguished from other types of tissues by their capacity for relatively unlimited and uncontrolled growth in the organism. The reason why neoplastic tissues grow continually, in contrast to embryonic tissues which grow only up to a certain point before they begin to differentiate, is a fundamental problem of cancer research. A study of the synthetic mechanisms of the cell is a logical approach to this problem. In this connection the nucleic acids have a special significance since the measurement of these compounds by means of ultraviolet spectrophotometry has demonstrated fluctuations in the nucleic acid content of the nucleus during the mitotic cycle and high concentrations of nucleic acid in the cytoplasm of cells which are actively dividing or which are engaged in protein synthesis (2-6).

Although the spectrophotometric method is extremely sensitive and permits the measurement of the nucleic acids in small portions of single cells, the results of such measurements are open to criticism on the ground that the method is not sufficiently specific. The results obtained by the physical method can, however, be tested experimentally by less sensitive but more specific chemical methods. Recently a method for the extraction of the nucleic acids from animal tissues and their estimation by colorimetric reactions was described (13) and the nucleic acid contents of homologous normal and cancer tissues were determined (14). It was found that the cancer tissues contained much higher concentrations of nucleic acids than did the normal tissues. In the present report, the results have been extended to include a larger number of normal and malignant tissues.

MATERIALS AND METHODS

Tissue preparation.—The tissues were removed as rapidly as possible from animals killed by decapitation and were homogenized in ice-cold distilled water in the apparatus of Potter and Elvehjem (11). The normal rat tissues were obtained from adult Sprague-Dawley rats. With the exception of the mammary tumors and the hepatomas, the cancer tissues were obtained from transplants which have been carried through a number of generations in this laboratory. The cancer tissues were obtained through the courtesy of Drs. J. A. and E. C. Miller, and B. E. Kline.

Analytical methods.—Aliquots of the homogenates were pipetted into centrifuge tubes, and acid-soluble, lipid, nucleic acid, and "protein" phosphorus were extracted and determined as described previously (13).[1] Desoxypentose nucleic acid (DNA) and pentose nucleic acid (PNA) were estimated in the nucleic acid extract by pentose determinations (13).

RESULTS

The results of the analyses are presented in Tables I and II. The amounts of DNA and of PNA phosphorus calculated from pentose measurements and the amounts of total phosphorus found in the nucleic acid extracts are reported in Table I. The ratios of the nucleic acid phosphorus calculated from pentose measurements to the total phosphorus found in the nucleic acid extract are also given in this table. This ratio should be 1.00, and from the data in Table I it is evident that the ratio is close to the theoretical for most of the tissues studied. Some of the analyses do, however, deviate from the ideal ratio. The reason for these deviations is not known, but similar discrepancies have been observed in previous work (13, 14).

Although the PNA content of the normal rat tissues varied considerably, the four types of rat tumors studied contained about the same amounts of PNA. Similarly, the DNA contents of the cancer tissues were fairly constant while the DNA contents of the normal rat tissues showed considerable variations. The nucleic acid contents of the mouse tumors were

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[1] In the removal of lipid phosphorus, the extractions with boiling alcohol-ether (13) were omitted since it was found that these steps did not materially increase the yield of extracted phosphorus.

also relatively constant, although the DNA levels were considerably higher than in the rat tumors.

Table II reports the acid soluble, lipid, and "protein" phosphorus contents of the tissues studied. It is evident that the amounts of these compounds found in the neoplastic tissues were in the range covered by the normal tissues. The lower content of these materials in the hepatomas as compared to the normal livers confirms previous observations (14). A study of studies and of studies on embryonic liver which showed that the enzymatic pattern of hepatomas resembled that of fetal liver more than that of normal liver (9, 12), it was concluded that cancer tissue probably represents a reversion to a more primitive type of metabolism (9). The nucleic acid data extend the enzyme results, since these data suggest that cancer tissue may represent a more primitive type of tissue in ways other than enzyme pattern. It will of course be

TABLE I: THE NUCLEIC ACID CONTENT OF NORMAL AND MALIGNANT TISSUES

Average values are given, with ranges of values in parentheses.

Tissue	No. of analyses	Mgm. phosphorus per 100 gm. fresh tissue			$\frac{P_{DNA} + P_{PNA}}{P_{total}}$
		PNA*	DNA*	Total phosphorus (found) in nucleic acid extracts	
Rat skeletal muscle	11	6.7 (4.7–10.4)	5.7† (3.6–9.4)	15.7 (10.0–20.8)	—
Rat cardiac muscle	12	12.4 (9.6–15.3)	14.5 (10.8–19.7)	33.1 (21.8–42.8)	0.81 (0.60–0.96)
Rat brain‡	3	17.5 (14.7–19.8)	12.3 (11.9–12.9)	29.5 (28.0–31.5)	1.01 (0.97–1.04)
Rat lung	9	18.0 (11.7–24.4)	60.5 (46.6–80.7)	78.5 (56.6–101)	1.00 (0.86–1.09)
Rat kidney	6	27.2 (24.9–30.2)	37.9 (33.1–42.6)	67.6 (58.5–73.0)	0.96 (0.90–1.04)
Rat thymus	8	37.8 (28.6–47.2)	264 (240–309)	298 (251–352)	1.01 (0.91–1.12)
Rat spleen	5	42.5 (36.7–53.7)	129 (115–135)	150 (135–168)	1.14 (1.09–1.24)
Rat liver	6	63.4 (55.0–71.5)	25.4 (20.8–31.3)	90.7 (78.0–104)	0.98 (0.78–1.23)
Rat pancreas	10	179 (147–234)	45.2 (37.2–48.2)	250 (203–280)	0.90 (0.71–0.99)
Rat hepatoma§	9	54.1 (32.9–69.1)	66.7 (48.0–84.9)	122 (91.9–152)	0.99 (0.87–1.12)
Flexner Jobling rat carcinoma	9	49.6 (35.2–64.0)	56.9 (47.2–62.6)	114 (93.6–133)	0.93 (0.86–1.09)
Jensen rat sarcoma	7	53.2 (47.0–59.2)	66.3 (52.0–75.9)	126 (111–137)	0.95 (0.83–1.02)
Walker No. 256 rat carcinosarcoma	5	59.5 (52.4–65.0)	66.3 (61.3–72.1)	131 (118–146)	0.96 (0.83–1.03)
Spontaneous mouse mammary tumors	6	59.6 (43.4–71.7)	104 (85.5–122)	159 (110–190)	1.03 (0.94–1.17)
U. V. mouse ear tumor	5	56.3 (49.8–64.7)	75.0 (62.8–88.7)	137 (115–151)	0.96 (0.94–1.01)
Mouse lung tumor‡	7	76.0 (59.1–85.5)	93.6 (65.4–109)	152 (120–174)	1.12 (1.03–1.26)

* Phosphorus calculated from pentose measurements (13).
† The DNA content of skeletal muscle was too low to be measured accurately and the figures stated are to be regarded as provisional.
‡ These data were reported previously (13, 14).
§ Induced by the ingestion of *p*-dimethylaminoazobenzene.

the individual compounds in each fraction will undoubtedly serve to emphasize any differences that may exist between these fractions in the normal and in the cancer tissues.

DISCUSSION

The observation that the different rat and mouse neoplasms have approximately the same content of PNA and of DNA is of considerable importance in view of enzyme studies which have demonstrated that the enzyme activities of different cancer tissues are relatively constant (8, 9, 16). As a result of these necessary to obtain nucleic acid data on fetal liver before it is possible to decide this point.

The nucleic acid contents of the tumors were found to be considerably higher than those of most normal tissues although some of the normal tissues contained more DNA or PNA than did the cancer tissues. It is of interest to note that tissues which are required to do large amounts of work continually, such as cardiac and skeletal muscle, contain very small amounts of nucleic acids. Indeed in the case of skeletal muscle, the DNA content was so low that it was impossible to

make accurate measurements. On the other hand, tissues such as liver and pancreas which are active in synthesizing materials contain large amounts of PNA. Thus the data in general confirm the results which Caspersson and his co-workers obtained with the spectrophotometric technic (2-6). Determination of the nuclear and cytoplasm volumes in the normal tissues and in the tumors would undoubtedly do much to facilitate a comparison of the data presented in this paper with that obtained by the physical method.

It is also of interest to compare the succinoxidase activities (16) of the normal rat tissues with their PNA contents (Table I) because of the question whether this enzyme system requires PNA for its activity (10, 15). If such a comparison is made, it at once becomes obvious that there is no correlation between the amount of PNA which a tissue contains and the succinoxidase activity of the tissue. For example, cardiac muscle possesses the highest enzyme activity of the tissues studied and an extremely low content of PNA, while in the case of tumor tissues the situation is reversed. It must be emphasized, however, that the entire evidence for or against the nucleoprotein nature of the succinoxidase system is indirect and that a final decision on this point will have to await isolation and purification of the enzymes involved.

The wide variations in nucleic acid content and the lack of correlation between nucleic acid phosphorus and enzymatic activity of normal tissues make the use of nucleic acid phosphorus as a basis for Q_{O_2} data (1) seem highly questionable. The use of nucleic acid phosphorus for this purpose was introduced into metabolic studies as a means of estimating the proportion of living cells in a tissue. In order for this method to be correct, the nucleic acids would have to be confined within the cells of the tissue and the nucleic acid content per cell would have to be the same in every tissue. The latter would seem to be highly improbable in view of the results presented in this paper. The use of nucleic acid phosphorus in metabolic studies is also complicated by the fact that the nucleic acid phosphorus always includes two variables, DNA and PNA, which apparently vary independently of each other in the different tissues. From these considerations, it would appear that the use of nucleic acid phosphorus as a measure of the cellular material in a tissue is unsatisfactory. The proportion of living cells in a tissue might be more satisfactorily determined by the direct method of Chalkley (7).

TABLE II: PHOSPHORUS COMPOUNDS IN NORMAL AND MALIGNANT TISSUES

Average values are given, with range of values in parentheses.

Tissue	No. of analyses	Mgm. phosphorus per 100 gm. fresh tissue		
		Acid soluble P	Lipid P	"Protein" P
Rat skeletal muscle	11	158 (138–180)	32.5 (26.4–36.9)	24.4 (19.3–29.4)
Rat cardiac muscle	12	91.6 (52.3–107.2)	81.7 (46.0–124.5)	41.7 (19.8–60.1)
Rat lung	9	54.8 (42.3–63.0)	88.6 (57.0–122.0)	24.7 (20.3–35.2)
Rat kidney	6	82.5 (78.3–91.0)	116.3 (95.0–143.0)	30.4 (19.5–45.7)
Rat thymus	8	88.3 (81.2–93.4)	56.9 (37.6–73.4)	45.3 (16.5–63.5)
Rat spleen	5	86.5 (82.8–90.3)	69.7 (53.9–84.3)	33.2 (21.7–53.9)
Rat liver	6	102.0 (96.8–111.5)	137.2 (98.4–169.7)	38.9 (28.3–54.4)
Rat pancreas	10	97.1 (86.9–108.0)	138.9 (101.2–177.7)	32.9 (26.9–45.8)
Rat hepatoma	9	94.2 (66.4–111.0)	79.3 (51.5–119.0)	24.3 (17.0–32.8)
Flexner Jobling rat carcinoma	9	75.2 (62.1–85.8)	54.4 (41.0–66.0)	23.0 (15.2–33.4)
Jensen rat sarcoma	7	76.2 (65.6–82.5)	55.5 (46.0–65.7)	25.2 (23.9–28.9)
Walker No. 256 rat carcinosarcoma	5	84.9 (75.6–88.9)	64.6 (58.1–81.0)	25.4 (16.5–32.2)
Spontaneous mouse mammary tumors	6	86.9 (57.3–109.0)	76.7 (52.6–111.1)	27.7 (20.5–33.5)
U. V. mouse ear tumors	5	89.0 (71.0–99.5)	53.8 (50.5–61.4)	31.4 (27.8–35.5)

SUMMARY

1. The desoxypentose nucleic acid, pentose nucleic acid, acid soluble phosphorus, lipid phosphorus, nucleic acid phosphorus, and "protein" phosphorus contents of several normal rat tissues and of several rat and mouse tumors were determined.

2. It was found that the desoxypentose nucleic acid and the pentose nucleic acid contents were relatively constant in the different neoplasms, while the nucleic acid contents of the normal cells varied considerably in the different tissues.

3. The acid soluble, lipid, and "protein" phosphorus contents of the neoplastic tissues fell within the range covered by the normal tissues. A study of the individual compounds that comprise these fractions will be necessary to decide whether any considerable differences exist between these fractions in the normal tissues and in the neoplastic tissues.

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Attempted Transmission of Acute Leukemia from Man to Man by the Sternal Marrow Route

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Previous attempts to transmit human leukemia from man to man with blood, cellular emulsions, from spleen and lymph nodules had completely failed. In these experiments the author (3) used the subcutaneous or intravenous route, with a subsequent period of observation of 24 months after the inoculations. With the subcutaneous or intravenous route of transmission the leukemic "agent" might not have come into contact with sufficient suitable and susceptible cells of the bone marrow. To place the material in contact with bone marrow cells the intrasternal marrow route of inoculation was chosen.

In the experiments described in this paper I used sternal bone marrow from untreated cases of acute leukemia for the transmissions. It was hoped to gain information about this special form of leukemia which differs from the chronic form by its rapid fulminant course, the age group involved, the specialized production of one cell-type only and the lack of response to any known treatment. The recipients of the leukemic material were human volunteers suffering from carcinoma of the oral cavity (tongue, pharynx, etc.). This did not exclude *a priori* the possibility of "takes," as the co-existence of leukemia and carcinoma in the same patient is known. Twenty such cases are recorded in the literature by Morrison, Feldman and Samwick (2), and by Berk and Movitt (1) who described a case of laryngeal carcinoma complicated by lymphoid leukemia. Two additional cases, one in a woman of 67 suffering from chronic lymphatic leukemia and sero-anaplastic carcinoma of the ovary, and one in a man of 56 suffering with chronic myeloid leukemia and a rectal adenocarcinoma, were observed in Adelaide.

SUBJECTS AND METHODS

Human volunteers who had been treated for squamous cell carcinoma of the tongue or pharynx were the recipients of the leukemic material. The diagnosis of carcinoma of the tongue was established by clinical examination and biopsy. The tumors were all treated with surgery and radium needling. Before the transmission attempts, the blood and sternal marrow of these cancer cases were examined. At the time of transmission 3 such cancer cases were placed on tables. The area over the manubrium sterni was anesthetized with 4 cc. of a 2 per cent novocain solution. A sternal puncture needle was inserted in each manubrium. Approximately 0.2 cc. of the marrow was aspirated for marrow films. The stiletto was then reinserted in the sternal puncture needle in the sternum.

Each case of acute human leukemia was prepared in the same way—a sternal puncture needle was inserted. Five cubic centimeters of the cellular sternal marrow were aspirated with a 10 cc. Record syringe. This material was injected through the already inserted needles into the sternal marrow of the recipients within a few seconds, and without any anti-coagulant, each recipient receiving approximately 1.5 cc. of leukemic bone marrow. The last drops of the aspirated leukemic material were used for marrow films and bacteriological examinations. Blood plates inoculated with sternal marrow from all 4 donors were found to be sterile after incubation for 48 hours. This procedure was repeated 4 times. The progress of the recipients was closely watched clinically and hematologically. At first daily, then weekly, later monthly blood counts, and also sternal marrow films at longer intervals, augmented the clinical examinations. In case of death of the patients with acute leukemias or of the recipients, complete or partial necropsies were performed.

The *first donor* was a boy of 10. He came to the hospital with a history of a sore throat, headache and breathlessness. The patient had bleeding, spongy gums, fever, a severe anemia, a high leukocyte count and an enlarged painful liver. The spleen was not palpable. The patient died after 29 days in the hospital. A postmortem examination was refused by the relatives. The bone marrow of this patient was used for transmission on the third day of his hospitalization. The peripheral blood on that day showed only 6.24 gm./per cent hemoglobin, and a leukocyte count of 103,000 of which 91 per cent were myeloblasts.

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The sternal marrow reflected, with 90 per cent, the same predominance of myeloblasts.

Recipients.—(1) A man of 67, treated with radium needles 17 years ago for a squamous cell carcinoma of the lip, and 6 months ago for a squamous cell carcinoma of the tongue. The patient had received a course of deep x-rays to the neck 3 months prior to the sternal inoculation.

(2) Man aged 70 (Wassermann reaction positive). The patient was treated with radium needles 5 years ago for a squamous cell carcinoma of the tongue, had deep x-ray therapy to the neck 4 years ago and, finally, had a resection of the tongue 3 years ago (apart from anti-luetic treatment 5 years ago).

(3) Man aged 72 (Wassermann reaction positive). The patient was treated with radium needles for squamous cell carcinoma of the tongue 40 months ago followed by excision of the lymph nodes of the neck 3 years prior to the sternal inoculation. This patient developed a mild secondary anemia with an increased monocyte count in the peripheral blood within 15 months of the inoculation. On examination an adenocarcinoma of the rectum was found, confirmed by biopsy and treated with colostomy. The patient died 181 days after the sternal inoculation from his carcinoma without any indication of leukemic lesions in bone marrow or blood.

The first two cases are still alive and clinically quite well without signs of leukemic lesions in blood or bone marrow 35 months after the sternal inoculations.

The *second donor,* a young man of 16, came to the hospital with a history of gastritis. He suffered from spongy gums, increasing anemia, and high fever. The examination of blood and bone marrow showed a severe anemia with a high output of myeloblasts in the peripheral blood and in the bone marrow. The swollen gums were typical, the liver was enlarged, the spleen not palpable. The patient lived for 29 days and died from the anemia. The findings at necropsy confirmed the diagnosis. On the fifth day of his illness while in the hospital his sternal marrow was used for transmissions. The peripheral blood showed 4.68 gm./per cent hemoglobin, the leukocyte count was 11,200 of which 99 per cent were myeloblasts. The sternal marrow was fluid, very cellular, and contained only 5 per cent normoblasts and 91 per cent myeloblasts.

Recipients.—(1) A man of 45, treated 12 months ago for a squamous cell carcinoma of the tongue with biopsy and radium needling. The lymph nodes of the neck were excised 10 months ago for a secondary deposit, followed by a course of deep x-ray 2 months later. For the last 8 months the patient was without further noticeable recurrences.

(2) A man of 88, treated with radium needling and deep x-rays for a squamous cell carcinoma of the tongue 3 years ago. The patient had secondary deposits in the lymph nodes of the neck, removed 13 months ago.

(3) A man of 67, treated with radium needles for squamous cell carcinoma of the tongue 15 months ago, followed by a course of deep x-rays to the neck 9 months later and excision of the lymph nodes of the neck a week prior to the inoculation.

Blood and bone marrow of these 3 recipients before and up to 26 months after the inoculation gave no evidence of leukemic lesions, progressive anemia, splenic or lymph node enlargement, or lesions of the gums.

The *third donor,* a man of 41, came to the hospital with dental symptoms which had persisted for 3 weeks. On examination he had ulcerated spongy bleeding gums, a swinging temperature, an enlarged liver, a string of palpable lymph nodes in the neck, a progressive severe anemia and an increased leukocyte count. The patient died after 36 days in the hospital and at necropsy had the typical lesions of an acute myeloblastic leukemia, involving gums, lymph nodes, liver, spleen and bone marrow. The sternal marrow of this case was used for inoculations on the 19th day of hospitalization. The peripheral blood showed 5.3 gm./per cent hemoglobin, 24,000 leukocytes with 60 per cent myeloblasts. The bone marrow also showed a marked predominance of myeloblasts.

Recipients.—(1) A man of 52, treated for carcinoma of larynx 8 months ago with deep x-rays, treated for carcinoma of the tongue with radium needling just prior to treatment and 20 days after the sternal inoculation. Later, recurrences and secondary deposits of his carcinoma of larynx and tongue appeared. The patient died 108 days after the sternal inoculation from hemorrhages from his ulcerated throat studded with necrotic cancerous material. At necropsy no evidence of leukemic lesions was found in the internal organs.

(2) A man of 52, treated with radium needles 14 months ago for squamous cell carcinoma of epiglottis at the base of the tongue, followed by a course of deep x-rays to the neck 8 months prior to, and 10 days after, the intrasternal inoculation with leukemic material. This patient died 217 days after the inoculation from an aspirative pneumonia due to an ulcerated carcinomatous nodule in his throat. At necropsy no evidence of leukemic lesions was found in the internal organs.

(3) A man of 77, treated with radon needles 13 days after the sternal inoculation for a squamous cell carcinoma of the tongue extending to the floor of the

TABLE I: LEUKEMIA DONORS

Name	Preclinical history	Total duration of illness in days	Day of donation	Blood count (day of donation) Group	Hemoglobin	White blood cells	Differential Cells	%	Bone marrow (day of donation) Differential Cells	%
(Male, aged 10 years) A. R.	Sore throat Breathless Headache 14 days	43	3rd	O (IV)	6.24	103,000	Polymorphs	2	Myelocytes	1
							Myelocytes	4	Myeloblasts	90
							Myeloblasts	91	Normoblasts	9
							Normoblasts	3		
							Anisocytosis and macrocytosis of red blood cells			
(Male, aged 16 years) K. F.	Stomatitis 3 weeks	29	5th	B (III)	4.68	11,200	Polymorphs and Eosinophils	1	Polymorphs	2
							Myeloblasts	99	Lymphocytes	2
							Normoblasts present		Myeloblasts	91
							Platelets scanty		Normoblasts	5
(Male, aged 41 years) W. G.	Dental symptoms 3 weeks	57	19th	A (II)	5.3	24,000	Polymorphs	12	Polymorphs	3
							Lymphocytes	20	Myelocytes	4
							Monocytes	4	Myeloblasts	78
							Normoblasts	2	Lymphocytes	5
							Myelocytes	2	Normoblasts	10
							Myeloblasts	60		
(Male, aged 21 years) P. B.	Ulcerated gums Sore throat	90	36th	O (IV)	11.7	347,000	"Blasts"	97	Polymorphs	3
							Myelocytes, Myeloblasts, Normoblasts	3	Metamyelocytes	1
									Myelocytes	1
									Myeloblasts	2
									Lymphoblasts	90
									Normoblasts	2
									Erythroblasts	1
									Eosinophils present	

mouth, and 5 months after with a course of deep x-rays to the neck. This patient is still alive after 24 months and without symptoms or lesions in blood or bone marrow which could be related to leukemia.

The *fourth donor* was a man of 21 with a history of a sore throat and agranulocytosis for 10 days. The patient arrived in the hospital with ulcerated gums, a painful liver and spleen, a moderate anemia and enlargement of the lymph nodes in the supraclavicular fossa. Sternal marrow was used for transmission on the 36th day of illness. The blood showed hemoglobin of 11.7 gm./per cent, a leukocyte count of 347,000 with 97 per cent of naked nuclei of the "blast" series. The bone marrow on that occasion was very cellular and 90 per cent of all nucleated elements consisted of these primitive "blasts." The patient died 90 days after admission to hospital with anemia, enlarged spleen, liver, large mediastinal tumor and generalized enlargement of lymph nodes. The microscopical examination of liver, bone marrow, lymph nodes and mediastinal tumor left no doubt that this case was an acute lymphoid and not a myeloblastic leukemia.

Recipients.—(1) A man of 48, treated with radon needles for squamous cell carcinoma of the tongue 5 months prior to, and with excision of the lymph nodes of the neck 20 days after, the sternal inoculation. The patient is apparently quite well 21 months after the sternal inoculation. There is no evidence of leukemic lesions in blood, or bone marrow.

(2) A man of 70, treated with radon needles for a squamous cell carcinoma of the tongue 74 months prior to, and with excision of the glands 20 days prior to, the sternal inoculation. This patient is also apparently quite well 21 months after the sternal inoculation without any indication of leukemic lesions.

(3) A man of 67, treated with radium needles for a squamous cell carcinoma of the tongue 11 months prior to, and with excision of the lymph nodes of the neck 11 days prior to, the sternal inoculation. This patient died 101 days after the implantation with a recurrent carcinoma and aspirative pneumonia. At autopsy no evidence of leukemic lesions was discovered in the internal organs.

Details relating to the leukemia donors are summarized in Table I.

RESULTS

No evidence could be detected clinically or hematologically that the implantations of leukemic bone marrow had any effect whatever on the recipients. No abnormal course or complication of the recipients' carcinoma, no change in the recipients' temperature, lymph nodes, spleen, leukocytes, hemoglobin or bone marrow could be detected during the time of observation which could possibly be attributed to leukemia. In no instance could immature leukocytes be detected in the peripheral blood. The percentage of myelocytes and especially myeloblasts did not increase in the bone marrow in any of the recipients. The hemoglobin and total leukocyte counts varied slightly. In every instance secondary deposits of the pre-existing carcinoma undergoing necrosis with sloughing or other complications such as aspirative pneumonia or hemorrhage were responsible. The findings of the repeated blood and bone marrow examinations were tabulated, but as only insignificant changes were observed this large collection of data is being omitted from publication.

It is obvious that the transmission attempts failed. However much the acute and chronic forms of leukemia differ clinically, both are apparently not transmissible under the circumstances described. Human leukemia resembles very closely the leukemia in mice and birds. In mice inbred strains are required for successful transmissions, but this is not so for birds. The lack of transmissibility of acute human leukemia has its counterpart in animal leukosis. It is an indication that the acute cases are only special forms of the chronic ones. They are different clinical manifestations of the same process appearing at an earlier date with a more rapid course.

The lack of "takes" in the described transmission attempts might be due to several factors such as the different genetic structure of the recipients from the donors, the age group of the recipients, their state of nutrition, and immunity to the implants due to the presence of a cancer. It is to be recalled also that as some virus-like agents, such as the milk factor, have an incubation period of about one-half the lifetime of the animal, it might be necessary to observe for a longer time than the periods recorded here, human subjects into whom leukemic material had been injected.

SUMMARY

Acute untreated human leukemia could not be transmitted with cellular sternal marrow by the sternal marrow route from man to man. No further evidence to separate acute and chronic leukemia, in spite of their clinical difference, could be brought forward. No evidence of a transmissible virus as cause of acute leukemia in man was detected.

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The Metabolism of 3,4-Benzpyrene into 8- and 10-Benzpyrenols in the Animal Body

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Peacock and his collaborators (12-15, 19) showed that injection of 3,4-benzpyrene into various animals caused a phenolic derivative to appear in the excreta. Further investigation by the present authors led to the recognition of 8-benzpyrenol in the feces of benzpyrene-injected mice and rats, associated with 3,4-benzpyrene-5,8-quinone (2, 3).

In order to provide larger amounts of benzpyrene metabolites,[1] and at the same time, to explore the possibility of species differences in the mechanism of metabolism, this work was extended to the rabbit. Preliminary investigations revealed the presence of metabolites other than those mentioned above, and a more extensive investigation was, therefore, undertaken to clarify the problem.

MATERIALS AND METHODS

More than 70 rabbits were used for this investigation. The animals were kept on a green-free diet for at least 3 weeks prior to the commencement of the experiment (*i. e.*, until the feces were free from chlorophyll and related colored substances that would have interfered with the subsequent chromatography), and the same diet was maintained during the experiment. This diet consisted of bread once a week, bran 3 times a week, oats 3 times a week, and water daily. Though such a diet may not be adequate for the rabbit when administered for long periods, no harmful effects could be detected during the relatively short period of the experiment.

Each rabbit received a single intraperitoneal injection of 5 ml. of a saturated solution of 3,4-benzpyrene in arachis oil (representing about 100 mgm. of benzpyrene per rabbit), and the feces were collected during the subsequent 2 to 3 weeks. The feces were dried in air, ground to a fine powder, and extracted with benzene by percolation at room temperature. The pooled benzene extracts were passed through columns of alumina (B.D.H.[2] Ltd.) for chromatographic separation. The appearance of the chromatogram, both in daylight and in ultraviolet light, are described in Table I.

TABLE I: APPEARANCE OF THE CHROMATOGRAM (ALUMINA COLUMN) FROM CRUDE FECES EXTRACT (BENZPYRENE-INJECTED RABBITS)

	Separation of zones	Appearance in daylight	Appearance in ultraviolet light (fluorescence)	Constituents present
	(Top of column)			
A	Narrow zone	yellow	faint greenish-blue	strongly adsorbed constituents of normal feces
B	Fairly wide zone	grey	violet	(?) probably contains derivatives of benzpyrene
C	Several narrow zones	brown; greyish-yellow	non-fluorescent	moderately adsorbed constituents of normal feces
D	Fairly narrow zones	greenish-brown	*strong bluish-white and greenish-yellow*	phenolic derivatives of benzpyrene
E	Diffuse wide zone	—	pale shiny greenish-yellow	mainly sterols; present in normal feces
F	Moderately wide zone	*orange-red with lower yellow border*	pink	quinone derivatives of benzpyrene
G	Very wide zone (extending to end of column)	—	strongly violet	unchanged benzpyrene
	(Bottom of column)			
H	Filtrate	pale yellow	strongly violet	unchanged benzpyrene

[1] For the purpose of investigating, by one of us (R.S.), their role in the mechanism of tumor inhibition by the parent hydrocarbon.

[2] British Drug Houses, Ltd.

The colored zone (F) and the strongly fluorescing zone (D) were separated by cutting the developed column, and were eluted—the former with chloroform and the latter with methanol. These were then followed up independently.

Colored fraction F.—The chloroform eluate was evaporated to dryness, and the residue dissolved in benzene and passed through a column of alumina. On development of the column with benzene, the red-colored zone displayed a yellow border at the lower end, which moved down on further development with larger quantities of benzene. Complete separation of the lower yellow from the upper red zone was not found possible even on prolonging the development for several days. But by repeated cutting and rechromatographing of the two extreme parts of the respective zones (*i. e.,* by concentrating on the upper parts of the red zone and the lower parts of the yellow, on each occasion), it was possible to obtain sufficient of the two components in a pure state for their identification.

From their colors, chromatographic behavior, and their color reactions with sulfuric acid, *i. e.,* olive green with the red, and cherry red with the yellow compound, respectively (20), it appeared probable that the red component was the 5,8-quinone, and the yellow component the 5,10-quinone of benzpyrene. This was confirmed by identifying the distinctive fluorescence spectra of their reductive methylation products as those of 5,8- and 5,10-dimethoxybenzpyrenes (2). As was to be expected, the reductive methylation product of the (mixed) middle zone provided a spectrum containing the bands of both compounds.

Fluorescent fraction D.—The fluorescent (phenolic) fraction of the original alumina column (fraction D), after elution with methanol and concentration *in vacuo,* was methylated with methyl sulphate and excess of NaOH.

Photographic examination of the fluorescence spectrum of this methylated product, elicited a complex band system (B, Fig. 1), strongly suggestive of a mixture of substances, since in addition to the bands of 8-methoxybenzpyrene, there were some in positions not previously identified.

Separation of the components was, therefore, attempted by the method of fluid chromatography. A solution of the methylated product in light petroleum was passed through a column of alumina, whereby all the material was adsorbed at the top. The column was then developed with light petroleum, followed by mixtures of light petroleum and benzene, and finally with benzene alone, the eluates being collected in separate lots, each of which was examined photographically for its fluorescence spectrum (C-F, Fig. 1). Those samples having identical spectra were recombined, and where resolution was evidently not satisfactory, the process of fluid chromatography was repeated.

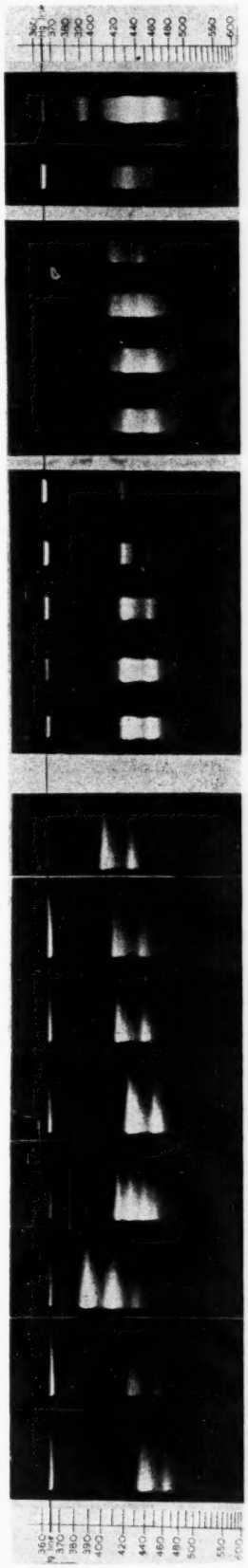
Complete separation of the unknown component from the 8-methoxybenzpyrene presented great difficulties, involving prolonged and repeated fluid chromatography, and entailed considerable losses of material. However, small amounts of the two separated components were ultimately obtained, by concentrating on the first few and the last few samples, respectively, from each chromatogram, the bulk of material remaining as mixtures in the middle fractions. The evidence, so far, pointed to the presence in the extracts of the rabbit's feces of at least 4 derivatives of benzpyrene, of which 3 (8-hydroxy-, 5,8-quinone, and 5,10-quinone) were known, while 1 was unknown. The unknown was phenolic in character, and probably a monohydroxy- derivative, as judged by the chromatographic behavior of its methylated product. (Dimethoxybenzpyrenes are more strongly adsorbed on alumina than monomethoxybenzpyrenes, and are, therefore, easily separated from 8-methoxybenzpyrene.)

Judging from the previous evidence (2) that the 5,8-benzpyrenequinone in mouse and rat feces was apparently derived from the phenolic metabolite (8-hydroxy-) by further oxidation (possibly by non-metabolic oxidation), an indication as to the probable position of the hydroxy- group in the unidentified compound could be inferred from the presence in the rabbit's feces extracts of the 5,10-quinone. Only the 5- or the 10- hydroxybenzpyrene could give rise to this product. The former was excluded, since the fluorescence spectrum of the unknown was slightly, but quite definitely, different from that of synthetic 5-methoxybenzpyrene (M, Fig. 1). (See also appendix for ultraviolet absorption spectrum.) There was, therefore, an indication that the methylated unknown rabbit metabolite was 10-methoxy-benzpyrene, and the metabolite itself, therefore, 10-benzpyrenol.

At the time these metabolic studies were in progress, 10-methoxybenzpyrene was not known. It has since been synthesized by one of us (R.S.) in collaboration with Prof. J. W. Cook, F.R.S.[3] Its fluorescence spectrum (N, Fig. 1) was found to be identical with that of the methylated unknown metabolite of the rabbit, and the same applied to their respective ultraviolet absorption spectra (See Appendix). Thus, the previous indication that the unknown metabolite was 10-benzpyrenol, received very strong support.

Though there was not enough of this metabolite for it to be identified by oxidation to the 5,10-quinone and the conversion of the latter into 5,10-dimethoxy-

[3] This synthesis will be described elsewhere.



A Methylated metabolite, mouse (in benzene)

B " " rabbit (")

C, D, E, F — Successive fractions of fluid chromatography of methylated *rabbit* metabolite (in mixtures of light petroleum and benzene)

G, H, I, J, K — Successive fractions of fluid chromatography of methylated *rat* metabolite (in light petroleum)

			Main band mμ
L	3,4-benzpyrene (BP)	(in liquid paraffin)	405
M	5-methoxy-BP (synthetic)	"	412
N	10-methoxy-BP (")	"	415
O	8-methoxy-BP (methylated metabolite)	"	426
P	Chromat. fraction of methylated rat metabolite	"	415 426
Q	Fraction V (from rat metab.)	"	389
R	5,10-dimethoxy-BP (synthetic)	"	425
S	5,8-dimethoxy-BP (")	"	437

FIG. 1.—The "peak" spectra (L-S) were obtained by the "moving edge" method (5).

benzpyrene, this procedure was carried out on the mixture containing the unknown together with the 8-methoxybenzpyrene. This yielded a product exhibiting the fluorescence bands of both the 5,8- and 5,10-dimethoxy- isomers.

The presence of 10-benzpyrenol and 5,10-benzpyrenequinone, besides the 8-benzpyrenol and 5,8-benzpyrenequinone in the rabbit feces, seemed at first to indicate a species specificity in the metabolism of benzpyrene, analogous to that observed with other hydrocarbons (7-11, 16, 17). However, inspection of the earlier plates containing the fluorescence spectra of benzpyrene metabolites of rats and mice (See A, Fig. 1), suggested, in the light of the newly-acquired spectrum of synthetic 5,8-dimethoxybenzpyrene, prepared from carefully purified 5,8-quinone (See S, Fig. 1) and those shown in the previous publication (2), makes it clear that the initial small peak in the spectra of the latter (both in the case of the synthetic and metabolic products) was in fact due to small traces of 5,10-dimethoxybenzpyrene; and similarly, that the slight fluorescence immediately preceding, and merging with, the major band of 8-hydroxy- (and 8-methoxy-) benzpyrene (A, Fig. 1) was due to traces of the 10- isomer (Fig. 2).

The presence of other metabolites.—The additional fluorescence in the region of 390 mμ in the original 8-hydroxy- (and 8-methoxy-) benzpyrene (A and P,

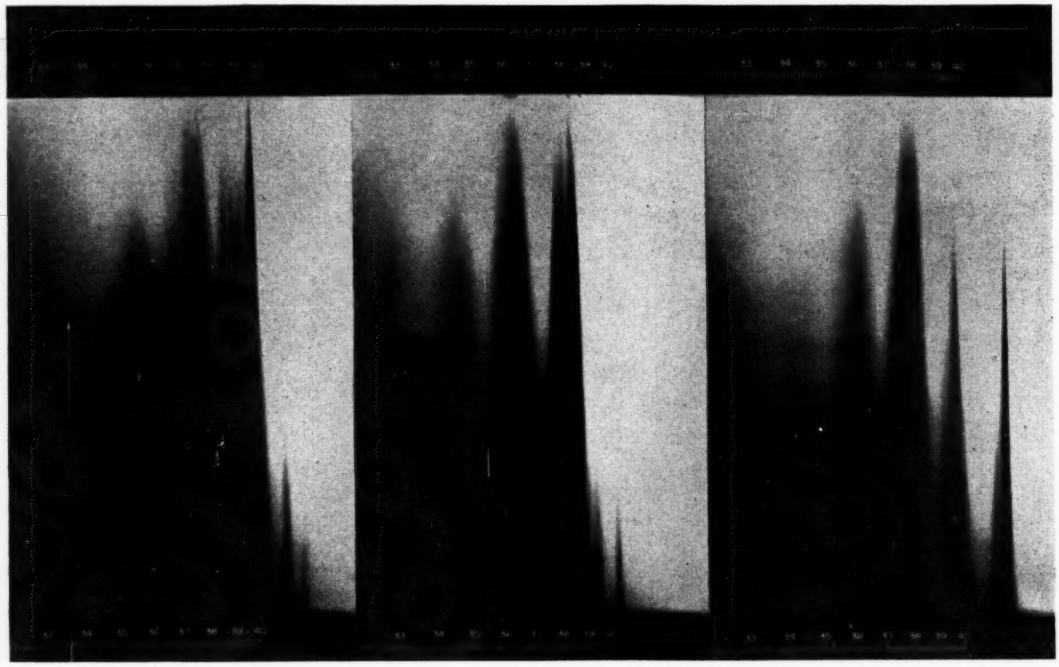


FIG. 2.—Spectrograms of the long wave band systems of 10-methoxy-3,4-benzpyrene, 3,4-benzpyrene, and 8-methoxy-3,4-benzpyrene, all in hexane.

experience, that the metabolism of these animals needed reinvestigating.

Reinvestigation of benzpyrene metabolism in rats.—Thirty rats were injected intraperitoneally with a saturated solution of benzpyrene in arachis oil (*i.e.*, about 40 mgm. per rat), and the feces collected and worked up in the usual manner. Careful chromatography of the methylated phenolic fraction revealed the presence of 10-methoxybenzpyrene in the rat as in the case of the rabbit (G-K, and P, Fig. 1); while a careful follow-up of the colored zones revealed also the presence of 5, 10-benzpyrenequinone. However, the relative proportion of the 10- and 5,10- to the 8- and 5,8- isomers, respectively, appeared to be lower in the rat than in the rabbit extracts.

Moreover, a comparison between the fluorescence Fig. 1), to which attention was drawn in the previous publication (2), was at the time suspected as being due to an impurity. In the present reinvestigation of the problem, it was found possible, by careful, repeated chromatography, to separate this component (Q, Fig. 1) from the methylated mixture, both in the case of the rat and the rabbit extracts. There seems little doubt that this product (called 'fraction V' in Fig. 1) is also derived from the injected benzpyrene.

However, in contrast to all the substituted benzpyrene derivatives so far studied, product V exhibits a fluorescence spectrum *shifted to the shorter wave length* as compared to that of benzpyrene (Compare Q with L-O, Fig. 1). While this might conceivably result from partial de-aromatization, or actual break-up, of the molecule of benzpyrene, further work is

needed to prove the point. Its absorption spectrum (See Appendix) is also very different from any of the known benzpyrene derivatives, and suggestive of a compound of simpler structure. The true nature of product V has, however, yet to be determined.

The strongly adsorbed fluorescent material (zone B, in Table I), which is as well considered to be derived from benzpyrene (21-23), was not investigated in detail.

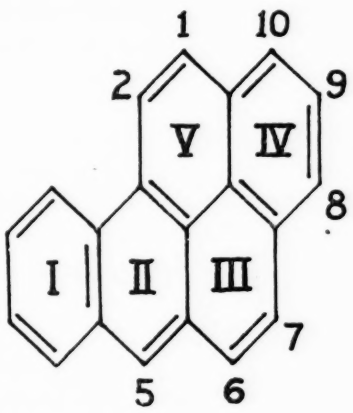
DISCUSSION

In any attempt to interpret the significance of the metabolic oxidation of 3,4-benzpyrene into its 8- and 10-hydroxy derivatives, the following facts have to be taken into account:

1. These positions in the benzpyrene molecule are not the ones which are chemically the most reactive (the latter being the 5- position), but represent the positions of secondary reactivity. In this respect, the metabolism of benzpyrene conforms to that of 1,2-benzanthracene (4), 1,2,5,6-dibenzanthracene (11), and probably of 9,10-dimethy1-1,2-benzanthracene (1). In the metabolism of chrysene (6), the position attacked (*i.e.*, 3-) is also not the most reactive, though whether it represents the position of secondary reactivity, has not yet been established.

2. Unlike the 5- position of benzpyrene, the 8- and 10- are both positions where the addition *e.g.* of H_2O_2 in the form of two OH groups to adjacent c atoms (called by Fieser "perhydroxylation") are possible, as is the case with the metabolites of the other polycyclic hydrocarbons so far studied. In the case of anthracene (7, 8), such an intermediate dihydro-dihydroxy-mechanism has actually been established, but the evidence in support of a similar mechanism in the case of 3,4-benzpyrene (21-23) does not seem to be conclusive.

3. Both the 8- and 10- positions of benzpyrene are alpha positions to angular double bonds (the 8-, in relation to the double bond 6,7-, the 10-, to the double bond 1,2-) corresponding to 1- positions in the phenanthrenoid parts of the molecule (rings II, III, IV and II, V, IV respectively). The same applies to the positions of metabolic oxidation of 1,2-benzanthracene (4), of 1,2,5,6-dibenzanthracene (11), and of chrysene (6).



3,4,-BENZPYRENE

There is as yet not sufficient evidence to say whether these three indications are inter-related. They may well point to the same underlying mechanism of metabolic oxidation for the whole group of polycyclic hydrocarbons.

SUMMARY

After intraperitoneal injection of 3,4-benzpyrene into rabbits, the feces were found to contain 8- and 10-benzpyrenols and 5,8- and 5,10-benzpyrenequinones.

A reinvestigation of the metabolism of benzpyrene in rats revealed the presence of the same products in their feces, though the relative amounts of 10-benzpyrenol and 5,10-benzpyrenequinone in relation to the 8- and 5,8- isomers, were less in the rat than in the rabbit extracts.

Evidence was obtained of the presence in rabbit and rat feces of other fluorescent products, probably derived from benzpyrene. Their nature has not yet been determined.

ACKNOWLEDGMENT

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Appendix

The Absorption Spectra of Some Methoxybenzpyrenes and of Methylated Derivatives of Metabolic Products of Benzpyrene*

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In a previous communication (1) the absorption spectra of synthetic 5,8- and 5,10-dimethoxy- derivatives of 3,4-benzpyrene and of a purified methylated metabolite of 3,4-benzpyrene (deduced to be 8-monomethoxybenzpyrene) were presented. Synthetic 5- and 10-methoxybenzpyrenes have now become available, the former prepared by Berenblum and his associates (1) by the method of Fieser and Hershberg (2), the latter by Cook and Schoental (See Fluorescent fraction D, above). In the present paper, the absorption spectra of 5-, 8-, and 10-methoxybenzpyrenes are reported, and are represented in Fig. 3.

The absorption spectra of two synthetic monomethoxybenzpyrenes (5-, and 10-), of two synthetic dimethoxybenzpyrenes (5,8- and 5,10-) and of one methylated metabolite (8-) whose structure has been established, are therefore now available for comparison with those of methylated metabolic products of benzpyrene investigated in the present work.

* This work was aided by maintenance and expenses grants from the Medical Research Council.

For this comparison, two methods have been used. First, the standard determination of the absorption curve by means of a Hilger Spekker photometer with a medium quartz spectrograph, using a spark between tungsten steel electrodes or a 108 watt Tungsten ribbon filament lamp as light source. Secondly, the moving plate device (3) adapted to the Hilger medium spectrograph, using a hydrogen discharge tube, or alternatively, the ribbon filament as light source. This latter method gives a high resolving power for fine structure bands in absorption spectra and, in the case of polycyclic aromatic hydrocarbons where 20 or 30 such bands are identifiable, affords a highly specific test of structural identity.

In the absorption spectra of the benzpyrene series of compounds examined here, 3 groups of bands are found. The long wave length group is the most sensitive to alteration in the position of substituents. In this group the long wave length bands are very sharply defined and therefore located with precision (± 0.15 mμ on the medium spectrograph). In spite of the low

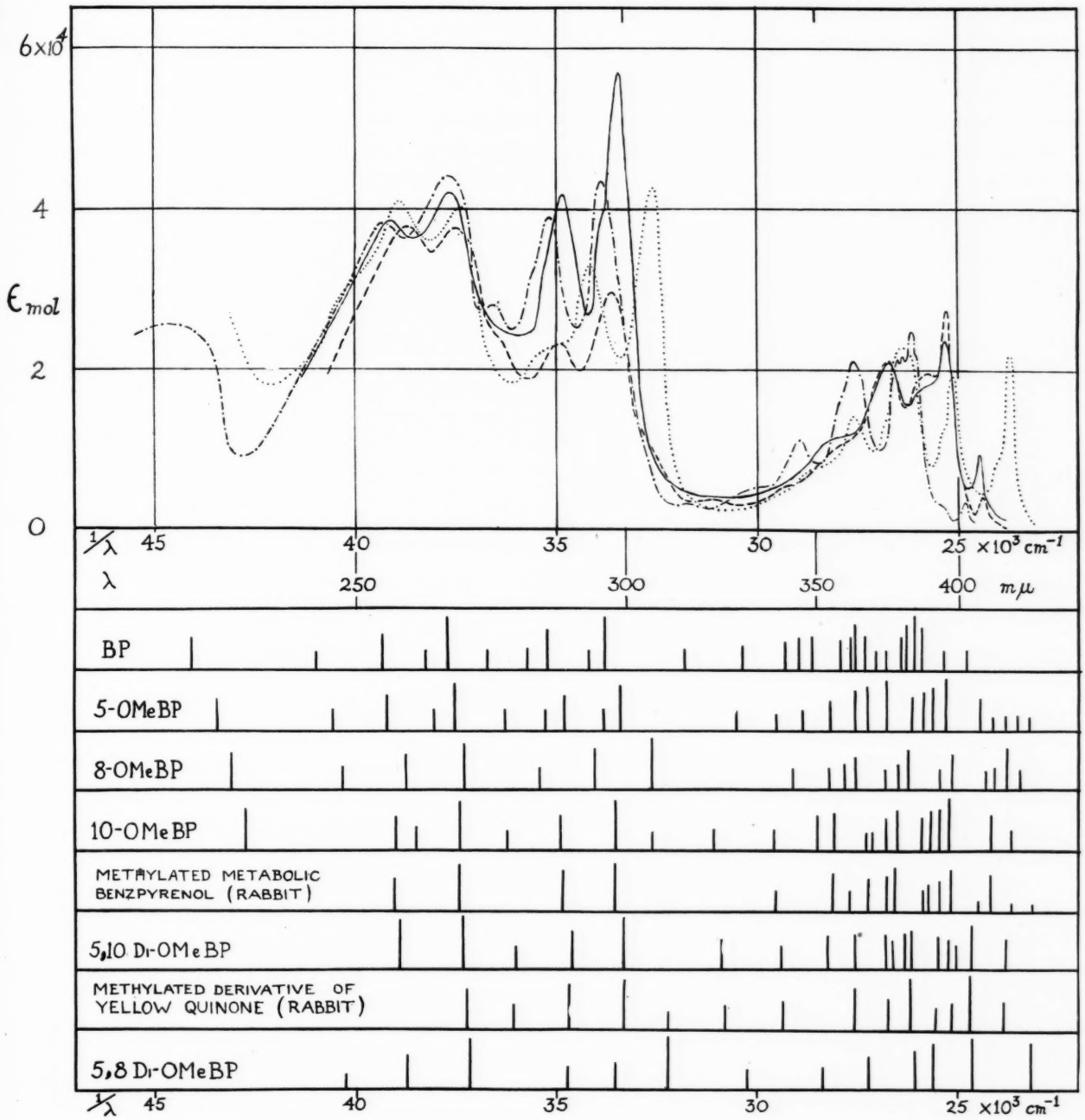

FIG. 3.—Spectral absorption curves of 3,4-benzpyrene (–·–·–·–·–·–·–), 5-methoxybenzpyrene (————————), 8-methoxybenzpyrene (. . . . .), and 10-methoxybenzpyrene (– – – – – – – –), all in hexane. The 5- and 10- were synthetic specimens; 8- was methylated metabolic product whose structure was deduced by Berenblum and his group (1). The absorption spectrum of 8- derivative is drawn to arbitrary vertical scale, as insufficient of specimen was available for accurate weighing.

Lower part of figure represents positions of fine structure bands of 3,4-benzpyrene, three mono-methoxy- and two dimethoxybenzpyrenes, and two methylated rabbit metabolites. Relative intensities of bands are indicated by heights of lines.

dispersion of the spectrograph at about 400 mμ, the favorable resolution of the fine structure bands obtainable with the moving plate method, as compared with the standard method, is shown by the fact that the bands of the long wave systems of the absorption spectra of benzpyrene and its derivatives are resolvable into as many as four bands by the former method whereas they are not resolvable by the latter.

An illustration of the type of spectrogram obtained by the moving plate method is given in Fig. 2 in which are shown portions of the spectrograms obtained with 3,4-benzpyrene and 8- and 10-monomethoxybenzpyrenes. In Fig. 3 are also shown the band positions of benzpyrene and five methoxy derivatives. These are compared with band positions of the two methylated benzpyrene metabolites isolated from both rabbit's and rat's feces. The first methylated metabolite has already been described (1) as 8-methoxybenzpyrene. The correspondence between the bands of the second methylated metabolite with synthetic 10-methoxybenzpyrene is close. All the high intensity bands agree in position. The fine structure of the absorption spectrum of the methylated metabolite is not quite so well resolved as that of the synthetic compound, some low intensity bands present in the latter are absent in the former, and there is an extra band in the spectrum of the metabolite at extremely long wave length (431 mμ). From these observations it is concluded that the metabolite is not in such a high state of purity as the synthetic compound.

The correspondence of the intensities and band positions between the 5,10-dimethoxybenzpyrene and the reductive methylation product of the metabolic yellow quinone is also very close. Again the resolution of the spectrum of the metabolite is not quite so good as that of the synthetic compound. Nevertheless it is evident that the methylated product of the metabolic yellow quinone is substantially 5,10-dimethoxybenzpyrene.

An examination of the absorption spectrum of fraction V (See *The presence of other metabolites,* above) showed no absorption above 390 mμ. This indicates the absence of the benzpyrene nucleus in fraction V. Several complex band systems were observed at wave lengths shorter than 390 mμ, which suggest a structure with a lower number of conjugated ring systems than in benzpyrene, possibly a phenanthrene derivative, which might have resulted from hydrogenation or break-up of two rings.

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Neoplasms of the Adrenal Cortex in Noncastrate Mice*

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Neoplasms of the adrenal cortex have appeared following gonadectomy in mice of both sexes of certain strains (2, 3, 10-17), and there were indications that such adenomas secreted estrogenic hormone.

Although the incidence of cortical adenomas was high in gonadectomized mice of these strains, similar tumors in noncastrate animals are apparently very rare (1, 8). Consequently, it was of interest to the authors that in the Minnesota line of the NH strain of mice [1] adenomas appeared spontaneously in a majority of female mice over 12 months of age. Although true carcinomas have not been observed thus far in females of this stock, two malignant adrenal cortical tumors have appeared in male animals, one in an intact NH mouse almost 2 years of age, a second in an F_1 hybrid male (NH × Strong A) which had received whole body irradiation with x-rays. These tumors were approximately one-third the size of the kidney. Cortical neoplasms also appeared in mice of other stocks which have been irradiated with x-rays. This subject will be discussed in a future communication.

MATERIALS AND METHODS

Zenker-fixed adrenal glands of untreated NH male and female mice of different ages were sectioned serially at 4 microns, every fifth section being mounted. Sections were stained with hematoxylin and counterstained with either eosin or Mallory's basic fuchsin. All female mice had been bred. The reproductive tracts were examined grossly and microscopically for evidence of the effects of secretion of sex hormones by the abnormal adrenal cortices.

RESULTS

NH female mice.—The adrenal glands of 14 female mice that were killed after one year of age were examined microscopically; 13 had adrenal cortical adenomas. Grossly these glands were in some instances merely nodular and only slightly enlarged, if at all, whereas in other cases the gland was from 2 to 4 times normal size. The glands of female NH mice were in general larger and whiter than those of males. In order to demonstrate adenomas histologically, serial sections of the adrenal glands were required. If an area of subcapsular hyperplasia invaded almost the entire thickness of the adrenal cortex, and "clear cells" as well as fibroblast-like elements were present, then the development was classified as an adenoma (Fig. 3). In several instances most of the adrenal cortex was replaced by adenomatous tissue (Figs. 6 and 10) although grossly the gland was but slightly enlarged.

Hyperestrinism was definitely demonstrated in one case. This was in a mouse 18 months of age. In spite of the obvious secretory inactivity of the ovary (Fig. 13), the endometrium was hypertrophic (Fig. 11) and the vaginal epithelium (Fig. 12) showed evidence of estrogenic stimulation. Fig. 10 indicates the appearance of an adrenal gland of this animal; most of the cortex was transformed into adenomatous tissue. The latter was histologically similar to the cortical alterations which have been described in castrate mice (14). Immediately beneath the capsule were basophilic fibroblast-like elements, the subcapsular "type A" (12) cells (Fig. 2). Among these there developed rosettes of the "clear" or "type B" cells (Fig. 4). Some spontaneous tumors were composed chiefly of subcapsular type A cells (Fig. 1), but in most instances rosettes of type B cells were found within the new growth.

Concerning the origin of the type A cells, it was the impression of the authors that they were derived from the capsule itself, rather than from cells of the zona glomerulosa. The type B cells represent differentiated type A elements. Regeneration of normal adrenal cortex from capsular elements has been described by several investigators (5, 9, 18).

NH male mice.—In 8 male mice over one year of age adenomas were not observed although subcapsular hyperplasia of type A cells (Fig. 5) was present. One spontaneous cortical tumor has been observed in a male mouse and this was larger (5 mm. in diameter) than any of the tumors that appeared in

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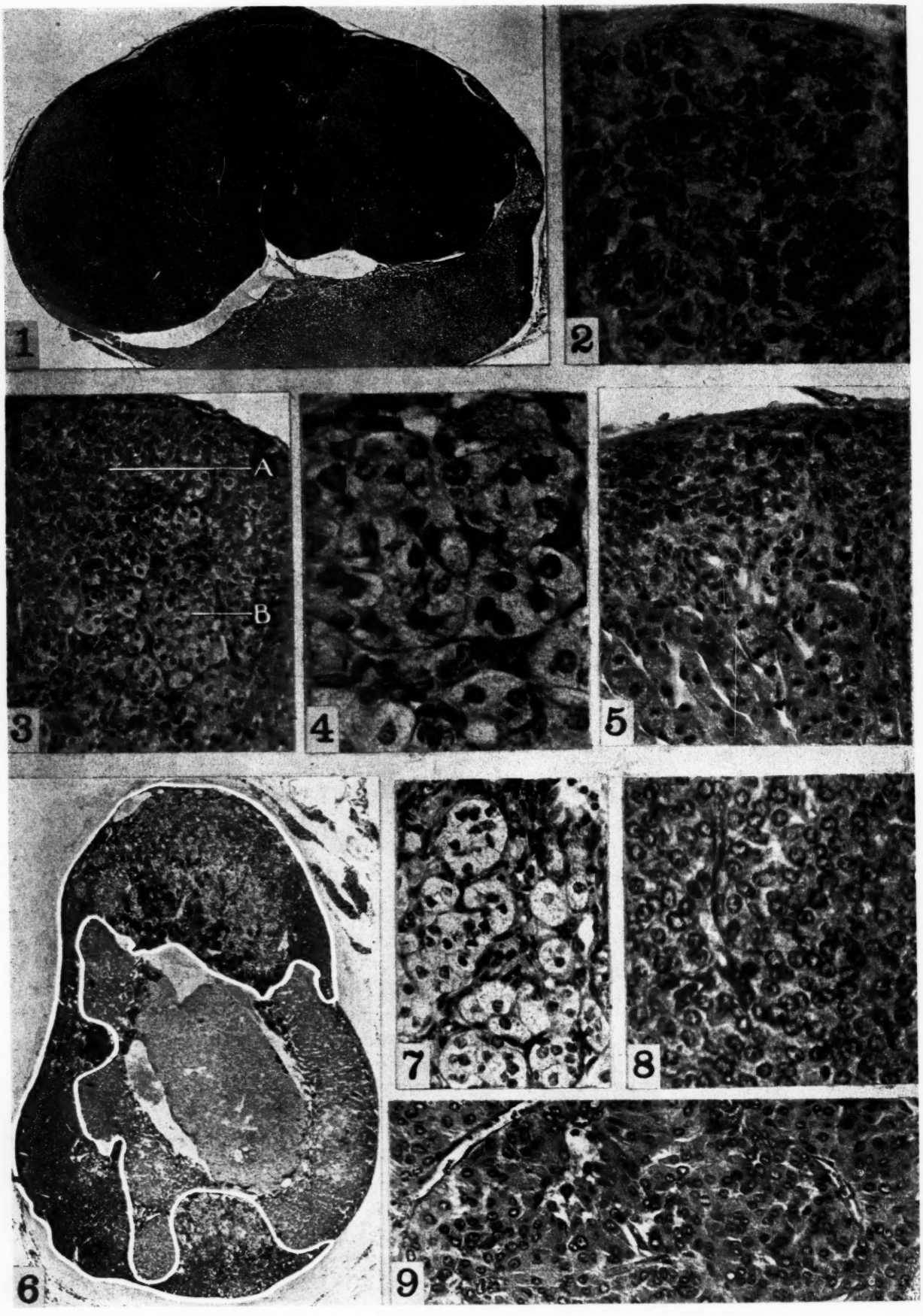
[1] This stock was obtained in the 8th inbred generation from Dr. L. C. Strong of the Yale University School of Medicine.



FIGS. 1–9

females. The tumor was composed of cords of undifferentiated cells which resembled type A cells to a greater degree than any other cortical cell (Fig. 8). The cytoplasm was more abundant, however, than in the type A cell of an adenoma, and there was a tendency towards the rosette formation which characterizes the "clear cells." Mitotic figures were present. Neither cortex nor medulla of the original adrenal gland could be found. Histologically this neoplasm might be classified as an adenoma rather than a carcinoma. It proved to be transplantable, and a growth 1 cm. in diameter was attained in the first transfer generation 6 weeks following transplantation. This tumor was similar in structure to the spontaneous cortical tumor reported in an intact female of the C strain (1).

Cortical carcinomas have been found in male mice irradiated with x-rays. One of these was in an F_1 hybrid cross between strains NH and Strong A that had received a total of 1,000 r of x-rays more than a year preceding autopsy. Sections are shown in Figs. 9 and 14. This tumor proved to be transplantable in F_1 hybrid mice of the same genetic constitution as the original host. This tumor and the neoplasm in the other NH male described above are classified as malignant on the basis of their microscopic structures. In neither host was there evidence of metastasis.

The germinal epithelium of both tumor-bearing male mice was spermatogenically active (Fig. 15). In the intertubular areas there were numerous "brown cells" which might suggest estrogenic secretion by these tumors; injections of estrogenic hormone into male mice result in the appearance of similar brown cells of the testis (4). Seminal vesicles were atrophic and did not evidence any effects of androgenic secretion.

DISCUSSION

These observations indicate that in the NH stock of mice gonadectomy is not necessary for the development of cortical neoplasms of the adrenal. They occur spontaneously in intact mice of both sexes. Animals bearing such tumors have all been beyond 1 year of age. Gonadectomy in this stock accelerates the onset of adenoma formation (6). Other adenomas which have been described following ovariectomy in the NH stock were in general larger than the spontaneous tumors reported here (3). They were also reported in older animals, and all of such tumors gave evidence of secretion of estrogen.

In most instances there was no evidence of pronounced estrogenic stimulation of the female reproductive tract in animals bearing spontaneous cortical adenomas. Further study is necessary to determine whether involution of uterus and vagina is delayed in this stock as compared with others that do not develop cortical adenomas. In one case hyperestrinism was demonstrated, indicating that spontaneous adenomas of the adrenal cortex, as well as those induced by castration, may secrete estrogenic hormone. Additional observations will be required to determine whether steroid hormones other than the estrogenic are produced by these adenomas.

The cells of the cortical adenomas which have been associated with the secretion of estrogen are the type B "clear cells." These possess a cytoplasm with little affinity for either acid or basic dyes; sparse acidophilic granulation appears in many of the cells. Similar cells can be seen occasionally in the ovaries of old mice of the NH stock (Fig. 7). The authors have studied an x-ray-induced ovarian tumor that was actively secreting estrogen and possessed similar clear cells.

The tendency towards development of adrenal cortical tumors following gonadectomy has been found to be present in stocks of mice with striking susceptibility (appearance of cancer in a high percentage of virgin females) towards the development of mammary tumors (10). This susceptibility was manifested only in the presence of the milk agent when adrenal adenomas served as the source of estrogenic hormone

DESCRIPTION OF FIGURES 1 TO 9

FIG. 1.—Spontaneous tumor of the adrenal cortex composed almost entirely of basophilic type A cells. From female NH mouse 16 months of age. Mag. × 40.

FIG. 2.—Type A cells from a spontaneous ademona occurring in a female NH mouse. Mag. × 250.

FIG. 3.—Spontaneous adenoma from adrenal cortex of female NH mouse. Note type A cells and rosettes of the type B "clear cells." Mag. × 160.

FIG. 4.—Type B "clear cells" from a spontaneous cortical adenoma. Mag. × 350.

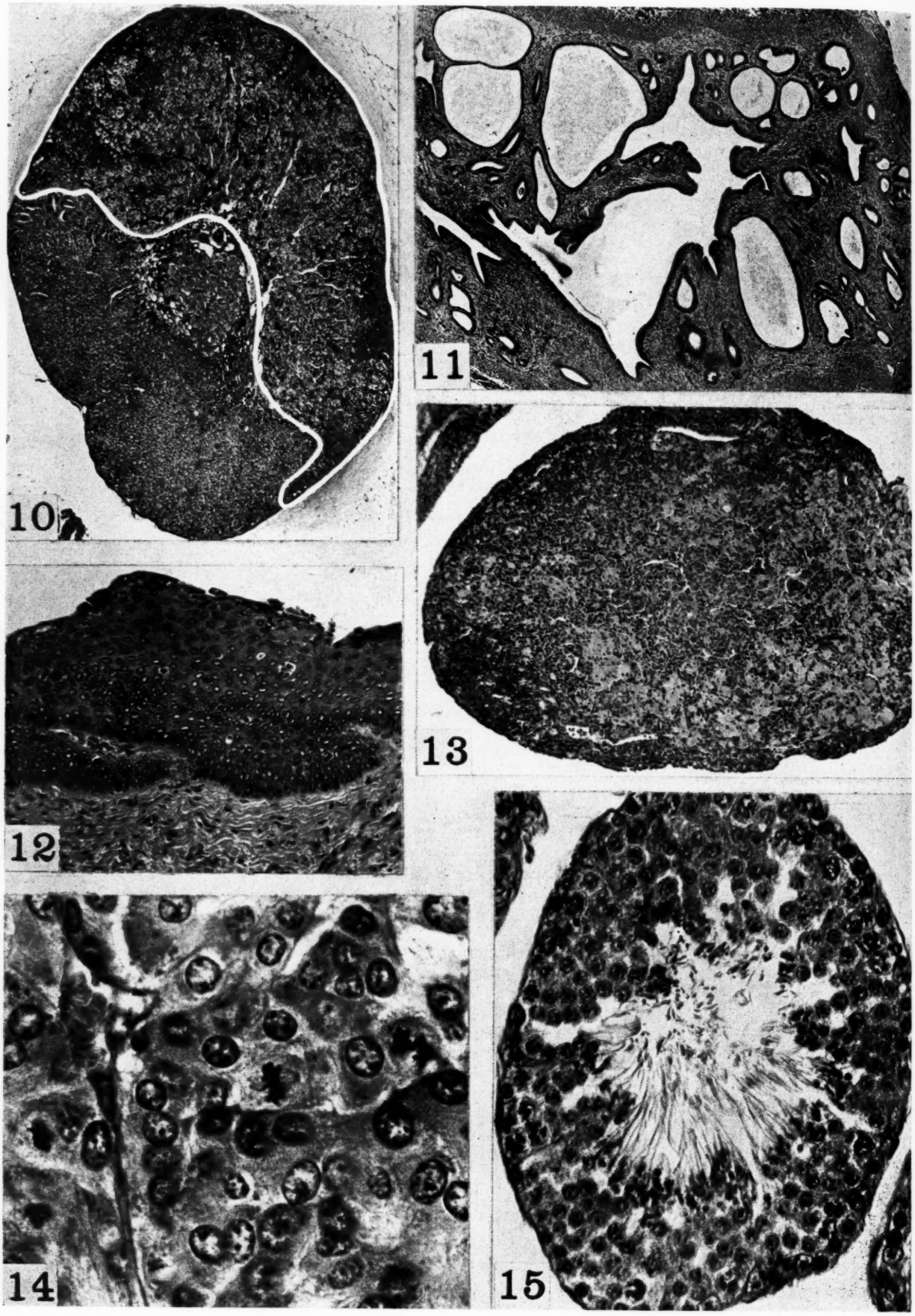
FIG. 5.—Hyperplasia of type A cells beneath the capsule of the adrenal of a male NH mouse 14 months of age. Mag. × 160.

FIG. 6.—Section through the adrenal gland of a female NH mouse 17 months of age. Adenomatous tissue of the cortex is outlined in white; remaining cortical tissue normal. The cells which photograph black are "brown cells" stained with basic fuchsin. Mag. × 40.

FIG. 7.—Cells of ovary from an old NH female. Note resemblance of these cells to the type B "clear cells" in Fig. 4. Mag. × 250.

FIG. 8.—Section from cortical neoplasm occurring in an NH male 23 months of age. The tumor cells resemble type A cells, although the cytoplasm is more abundant. Grossly tumor was 5 mm. in diameter. Mag. × 250.

FIG. 9.—Section of carcinoma of the adrenal cortex appearing in an F_1 hybrid male (strain NH crossed with Strong A strain). Mouse was 16 months of age and had been irradiated with 1000 r of x-rays in divided doses one year earlier. Mag. × 250.



FIGS. 10–15

in gonadectomized mice (7). The NH stock does not develop spontaneous mammary cancer (6), and reciprocal crosses have demonstrated that this stock lacks the milk agent (6). NH females might possess the "hormonal constitution" associated with pronounced susceptibility to mammary cancer development, but lack other factors necessary for the genesis of spontaneous mammary cancer.

Since adrenal cortical adenomas develop following castration, it might be inferred that the adenomas of the NH stock appear subsequent to the spontaneous cessation of gonadal activity. In the two cases of cortical tumor appearing in males, however, the germinal epithelium was active in the production of sperm (Fig. 15). These mice were not "physiologic castrates" from the standpoint of germ cell production, although the androgenic output of the testes was probably low as judged by the histology of the seminal vesicles. Androgenic secretion might have been counterbalanced, however, by the estrogen production of the tumors.

SUMMARY

In the NH stock of mice gonadectomy is not necessary to induce the development of neoplasms of the adrenal cortex. Adenomas of the adrenal cortex appeared spontaneously in 13 of 14 intact female mice over one year of age. In one instance the secretion of relatively large amounts of estrogenic hormone by such an adenoma was demonstrated. Cortical adenomas are probably relatively infrequent in male mice of this stock. Two histologically malignant adrenal cortical tumors found in male mice are described. The testes of both mice were spermatogenically active.

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DESCRIPTION OF FIGURES 10 TO 15

Fig. 10.—Cortical adenoma occurring spontaneously in an NH female mouse 18 months of age. Adenomatous tissue is outlined in white. Remainder of cortex normal. Mag. × 40.

Fig. 11.—Hypertrophic endometrium taken from same mouse whose adrenal is shown in Fig. 10. Cystic glands are indicative of hyperestrinism. Mag. × 90.

Fig. 12.—Vaginal epithelium from same mouse. Marked stratification of the squamous epithelium indicative of hyperestrinism. Mag. × 100.

Fig. 13.—Ovary of same mouse, tissues from which are shown in Figs. 10-12. Ovary composed of basophilic connective tissue cells and "brown cells," which are the large cells appearing light-grey in this photograph. Mag. × 40.

Fig. 14.—Section from carcinoma of the adrenal cortex appearing in F_1 hybrid male (NH × Strong A) which had previously been irradiated with x-rays. Mag. × 500.

Fig. 15.—Section through a seminiferous tubule of the NH male bearing the adrenal carcinoma pictured in Fig. 14. Note the active germinal epithelium with mature sperm cells. Mag. × 300.

Transplantation of an Adrenal Cortical Carcinoma*

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The occurrence of adrenal cortical carcinomas in gonadectomized JAX strain ce mice has been reported (3). Evidence was presented which indicated that the adrenal tumors were the source of internal secretions which were stimulating to the accessory sex organs. This report concerns transplantation studies with one of these tumors.

REVIEW OF LITERATURE

One reference to the transplantation of an adrenal cortical tumor of the mouse has been found in the literature. Dalton, Edwards, and Andervont (1) reported on a spontaneous, transplantable, adrenal cortical tumor in a female strain C mouse 24½ months of age. They presented evidence that this neoplasm originated from relatively undifferentiated cells of the adrenal cortex. The failure to find metastases from the original growth or the transplanted tumors suggested that the neoplasm was relatively benign in character. However, the rapid growth rate attained in later generations plus invasion of the capsule indicated a certain degree of malignancy. Careful examination for gross and histologic changes, particularly in the urogenital system and adrenal glands, was made. In female hosts focal edema was noted in the deeper layers of the endometrial stroma of the uterus; no changes were noted in the genital system of male hosts.

MATERIAL AND METHODS

This report is based on an adrenal cortical carcinoma P1699 arising in an ovariectomized ce strain mouse. Some data regarding the original tumor and its host have been included in earlier reports (3, 4). Tumor transplatation was into ce strain mice unless otherwise noted.

The tumor tissue was transplanted into four types of mice: (a) gonadectomized females, (b) gonadectomized males, (c) intact females, and (d) intact males. When gonadectomized mice were used, the gonads had been removed 1 to 3 days after birth.

* This work has been aided by grants to the Roscoe B. Jackson Memorial Laboratory from the Commonwealth Fund, The Anna Fuller Fund, The Jane Coffin Childs Memorial Fund for Medical Research and The National Advisory Cancer Council.

The mice were kept in compartments (6×12 inches) in groups of 2 or 3 to a compartment. They were fed Purina fox chow and water.

Transplantation was into young mice and autopsy was performed at various mature ages. (See Tables I, II, III, IV.) Following autopsy macroscopic as well as microscopic observations were made on many organs, including the submaxillary glands, mammary glands, seminal vesicles and prostates and, in the female, uterus and vagina. The mammary glands were fixed on the skin and examined in gross mounts.

The tumors were classified according to size as follows: Microscopic to 0.5 cm. in diameter (small); 0.5 cm. to 1.5 cm. in diameter (medium); greater than 1.5 cm. in diameter (large).

RESULTS

The adrenal cortical carcinoma P1699 has been described (2, 3) but a brief review is given here. The original mouse, an ovariectomized female 17 months of age at autopsy, had 2 adrenal tumors. The larger tumor, the one that was on the left side, was transplanted. Two main types of tumor tissue were present in the original tumor. These will be designated type I and type II as has been done in another report (5). Examples are shown in Figs. 1 and 2.

In the original host the submaxillary gland was not of female, but of male, type. The uterus was large in diameter. The epithelial cells lining the lumen of the uterus were tall columnar with centrally placed nuclei similar to (4) Fig. 3. This nuclear position was especially pronounced in the uterine glands. The vaginal epithelium was 2 cell layers thick and mucified, although only moderately mucified as compared to others in the ovariectomized series, that is, to (4) Fig. 6.

The mammary glands were of medium length and narrow when compared to those of ce strain virgin females of similar ages. No alveoli, or end buds, the latter indicative of active mammary gland growth, were present.

Ovariectomized female mice.—Adrenal tumor P1699 was transplanted intraperitoneally into 5 ovariectomized female mice when they were 21 to 39 days of age. In 39 day old mouse P2003 it was transplanted

subcutaneously. Autopsy was at ages ranging from 131 to 340 days; that is, the tumors were carried from 98 to 319 days. The transplants developed in 5 of the 6 mice. A summary of the data is presented in Table I.

The transplant consisted entirely of type I tumor tissue in 3 mice, a type characteristic of the major part of the original tumor. In 2 mice, P2003 and P2126, small areas of type II adrenal cortical tumor tissue were also present, as in the original tumor. These 2 animals were of the first and second transplant generation respectively.

The uterus of P2231 was not larger than that expected in a typical castrate. The epithelial cells lining the slit-type lumen were cuboidal. In three mice, P1874, P2003, and P2203, the uteri were larger than in the castrate type but were still small in diameter. The uterine glands were enlarged. In P1874 the cells lining the lumen were columnar of moderate height, with oval nuclei situated near the center of the cell. The cells were closely packed. The epithelial cells were low, columnar in P2003 and P2230. P2218, which carried the transplant for 319 days, had a uterus large in diameter and had well developed uterine glands. The cells lining the lumen were columnar, of moderate height, closely packed and had nuclei (in relation to the lumen) centrally placed.

The vaginal epithelium was observed in all the mice. In P1874 it was 4 to 6 cell layers thick and in addition there was a cornified layer. In P2003 the epithelium was two cell layers thick with some mucus. It was 3 to 4 cell layers thick in P2126 and 2 cell layers thick in P2230, P2231, and P2218. In P2230 the cells of the superficial layer were tall columnar. In P2218 the vaginal epithelium was heavily mucified.

The submaxillary glands were of male type in all mice except P2231. In P2231 they were in general of female type although in regions similar to that in a young male. It was classed as intermediate in type.

The mammary glands of the mice in this series were not well developed. Within individual mice they were uniform in development. In general the ducts were of medium length, narrow, moderately branched, and without end buds or alveoli. They were the best developed in P2218 which had carried the tumor transplant over the longest time and were the least developed in P2231 in which the transplant was negative.

P2231 in which the tumor transplant did not grow was the only mouse in the series with an adrenal cortical tumor in each adrenal. These were small but typical adrenal cortical carcinomas.

Among the 5 with successful transplants, 4 had no primary adrenal cortical tumors, although P2126 had an area of the type which has been interpreted as precancerous (2). P2230 had a tumor of the left adrenal about 7 mm. in diameter. It is probable that the transplant grew exceptionally slowly in this mouse as it was not noticed in palpating the animal 111 days after transplantation while in P2126 it was palpable at a similar age.

Table I: Adrenal Tumor P1699 in Ovariectomized Mice

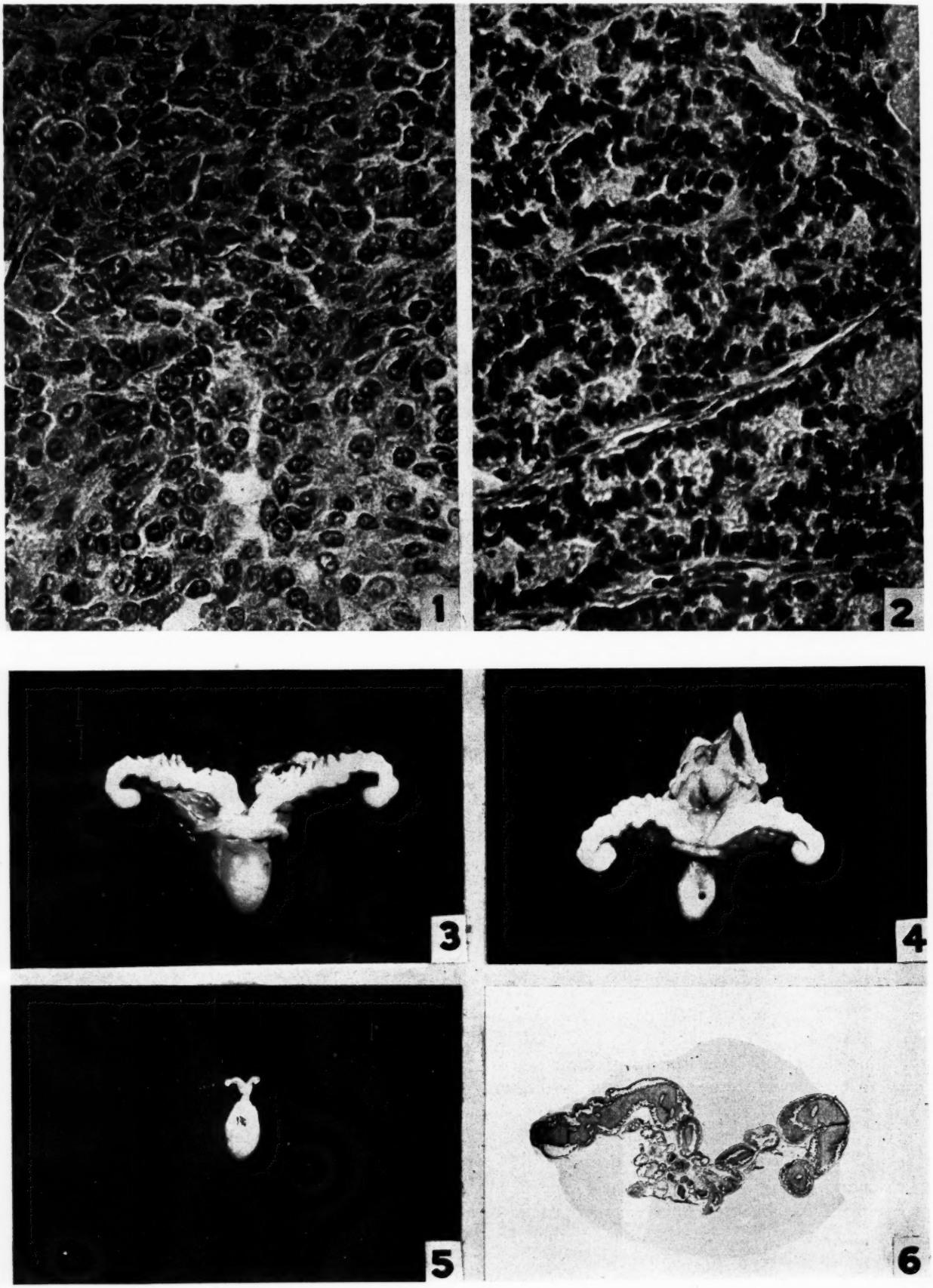
Mouse number	Generation of transplant	Days of age at transplantation	Days of age at autopsy	Size of transplant at autopsy	Diameter of uterus	Thickness of vaginal epithelium	Submaxillary gland	Primary adrenal tumors	Mammary glands
P1874	1	39	131	medium	small	cornified 5–6	♂-type	0	+
P2003	1	39	234	large	"	mucified 2	"	0	+
P2126	2	32	254	"	—	3–4	"	precancerous lesion	+
P2230	2	32	279	"	small	2	"	1	+
P2231	2	32	281	absent	"	2	intermediate	2	+
P2218	1	21	340	large	medium	mucified 2	♂-type	0	+

Mice with positive tumor transplants had very little adrenal cortical cell hypertrophy (type B cells). Extensive areas of type A cells were present.

Intact female mice.—The tumor was transplanted into 3 intact female mice. Data, including the age at transplantation and age at autopsy, are summarized in Table II. The transplanted tumor at autopsy was of medium or large size in all mice and was uniformly of type I tumor tissue. None of these mice had spontaneous adrenal cortical tumors.

In P1989 the uterus was normal in diameter and the uterine glands were normally enlarged. The epithelial cells lining the lumen were columnar and of moderate height. The nuclei were in a central position in the cells. The vaginal epithelium was 2 cell layers thick. The cells of the superficial layer were tall columnar. The submaxillary gland was male type. The mammary glands were similar to those of virgin females of similar age. The tumor had been transplanted into the abdomen of this mouse and at autopsy part of the tumor was growing within a lobe of the liver.

P2582 was injected subcutaneously with the tumor.



FIGS. 1-6

At autopsy the uterus was large in diameter and normal in structure. The epithelial cells lining the lumen of the uterus were columnar, of moderate height and the cell outlines indistinct. The vagina had an epithelial layer 10 to 12 cell layers thick and in addition a stratum corneum. There were a few leukocytes in the lumen. The submaxillary gland was typical female type. The mammary glands were similar to those of P1989. The ovaries, which were similar to those of P1989, had few follicles and many lipochrome cells, that is, giving the appearance of an inactive gland.

P2596 was injected subcutaneously with tissue obtained via ♀ P1989 and castrate ♂ P2395. The uterus was of medium diameter with shrunken stromal cells. The epithelial cells surrounding the lumen were cuboidal. The vagina was not examined. The submaxillary gland was female type.

Intact male mice.—The adrenal cortical carcinoma P1699 was injected subcutaneously in 12 intact male mice between the ages of 27 and 71 days. In all mice the transplanted tumor was of large size at autopsy. The hosts were then from 117 to 458 days of age (Table III). The tumor tissue was uniformly type I.

Gross and histological observations showed the mice to be not greatly changed over non-tumor intact male mice. The submaxillary glands were male type. The mammary glands were rudimentary. Evidence of spermatogenesis was observed in the testes. The adrenal glands were without cortical tumors. The seminal vesicles and prostates were large. However, in some mice with large tumors the seminal vesicles were not as large and turgid as in intact mice of similar ages without tumors.

Castrated male mice.—Adrenal cortical tumor P1699 was transplanted subcutaneously in 8 castrated males. The data concerning these mice are summarized in Table IV. In 5 mice the tumor was the second, and in 3 mice was the third generation transplant. Transplantation was done when the host mice were between 35 and 126 days of age. The host age at autopsy varied from 181 to 491 days. In all except the mouse autopsied at 181 days the transplants were large. In this one mouse the transplanted tumor was of medium size. In all mice the tumor tissue was type I.

The submaxillary gland was male type in all mice. The terminal tubules occupied a more extensive part of the gland in a number of animals than in normal intact male mice.

The seminal vesicles and prostates were enlarged as if stimulated in a manner similar to a normal male although in general they were not as large and turgid as in normal ce strain males. Fig. 4 shows P2314 as a gross specimen.

TABLE II: ADRENAL TUMOR P1699 IN INTACT FEMALE MICE

Mouse number	Generation of transplant	Days of age at transplantation	Days of age at autopsy	Size of transplant at autopsy	Diameter uterus	Thickness of vaginal epithelium	Submaxillary gland	Primary adrenal tumors	Mammary glands
P1989	1	21	182	medium	large	2	♂-type	0	+
P2582	3	22	185	large	"	cornified 10–12	♀-type	0	+
P2596	3	36	378	"	medium	—	♀-type	0	—

TABLE III: ADRENAL TUMOR P1699 IN INTACT MALE MICE *

Mouse number	Generation of transplant	Days of age at transplantation	Days of age at autopsy	Size of transplant at autopsy
P2539	4	27	117	large
P2559	4	27	140	"
P2567	4	27	153	"
P2412	3	47	191	"
P2409	2	30	211	"
P2508	3	68	219	"
P2587	4	27	236	"
P2507	3	38	360	"
P2501	3	54	384	"
P2540	3	38	406	"
P2493	3	71	408	"
P2541	3	54	458	"

* In all animals seminal vesicles and ventral prostates, large; submaxillary glands, male type; primary adrenal tumors, absent; and mammary glands, rudimentary.

DESCRIPTION OF FIGURES 1 TO 6

FIG. 1.—Type I adrenal cortical tumor tissue in P1699. Mag. × 500.

FIG. 2.—Type II adrenal cortical tumor tissue in P1699. Mag. × 500.

FIG. 3.—Accessory reproductive organs, seminal vesicles and ventral prostates of a mature strain ce intact male 8 months of age at autopsy. Slightly magnified.

FIG. 4.—Accessory reproductive organs, seminal vesicles and ventral prostates of a gonadectomized male P2314 bearing transplanted adrenal tumor P1699; stimulated to almost normal size. Same magnification as Fig. 3.

FIG. 5.—Accessory reproductive organs of a strain ce male before spontaneous adrenal tumor developed. Castrate condition. Same magnification as Figs. 3 and 4.

FIG. 6.—Section through accessory reproductive organs of castrate male P2492 bearing tumor P1699 for 171 days. Transplant adrenal tumor, large at autopsy. Mag. × 4.9.

The mammary glands were either absent or rudimentary in all mice. This is interesting in view of the fact that they were well developed in many mice with spontaneous adrenal cortical tumors (4, 5).

There were no spontaneous adrenal cortical tumors except in P2503. In the latter mouse the right adrenal had a cortical carcinoma 3 mm. in diameter. P2503 also varied from the other mice in this series in that transplantation was performed at a much older age, *i. e.,* at 126 days rather than at about 40 days. This mouse was also 125 days older at autopsy than any other mouse in this series.

Hybrid mice.—A transplant of the adrenal tumor grew successfully in a ♀ F_1 generation dba ♀×ce ♂ mouse. A transplant also grew successfully in a gonadectomized female F_1 generation C57 black ♀×ce ♂ mouse.

TABLE IV: ADRENAL TUMOR P1699 IN CASTRATED MALE MICE *

Mouse number	Generation of transplant	Days of age at transplantation	Days of age at autopsy	Size of transplant at autopsy
P2174	2	44	181	medium
P2492	3	42	213	large
P2255	2	40	215	"
P2538	3	35	252	"
P2312	2	40	296	"
P2314	2	40	296	"
P2395	2	44	366	"
P2503	3	126	491	"

* In all animals seminal vesicles and prostates, enlarged; submaxillary gland, male type; primary adrenal tumors, absent with the exception of P2503; and mammary glands, rudimentary.

DISCUSSION

An important fact revealed in the present study is that an adrenal tumor of the type occurring in ce strain mice following gonadectomy can be transplanted successfully generation after generation for at least 4 generations. Transplantation was possible not only in ce strain mice but in F_1 generation mice where a ce strain mouse was one of the parents.

By using the transplantation technic it may be possible to secure much information about ce strain adrenal cortical tumors. Some information has already been obtained using tumor P1699. The fact that the seminal vesicles and prostates were enlarged in gonadectomized male mice bearing tumors transplanted and also that the submaxillary glands in these mice and in 5 of 6 gonadectomized female mice were of male type indicates that the tumor was associated with internal secretions which were androgenic in effect. The fact that the mammary glands did not develop unusually in these mice indicated that very little estrogenic hormone was present. It is interesting that the mammary glands failed to develop because, although they were not greatly developed in P1699, they were extensively developed in some of the mice with primary adrenal cortical tumors (4). The vaginal epithelium was thickened and cornified in one ovariectomized mouse with the tumor transplanted.

The situation with the 3 intact female mice is interesting in that in 1 mouse the submaxillary gland was male type, and was female type in 2 mice. It is hoped that more experimental work will make the reason for this clear. It may be that the tumor changes under different influences. One possible factor was that it was observed that P1699 in common with many transplantable tumors grew more rapidly as transplant generations increased. The mouse with male type submaxillary gland was in the first transplant generation while the 2 with female type submaxillary glands were of the third transplant generation. Another difference was that the former was grown in the abdomen while the latter 2 were grown subcutaneously.

By transplantation, tumor tissue may be increased so that sufficient tissue might be obtained for detailed chemical analysis. It would also be possible to increase the number of animals with transplant tumors so that an analysis of body fluids of the mouse might be possible.

In earlier reports it has been shown that either the testes or the ovary prevents the occurrence of spontaneous adrenal cortical tumors in ce strain mice. Evidence is here presented that the adrenal tumor transplant tends to prevent the occurrence of these tumors. One primary tumor of the adrenal cortex appeared out of 4 ovariectomized mice of suitable age, with transplants, and 2 occurred in 1 mouse of a similar age in which the transplant was not successful. One primary tumor occurred in 7 castrated males interpreted to be of tumor age. Transplantation was done at a late age, 126 days, in the mouse with a primary tumor.

SUMMARY

An adrenal cortical tumor, P1699, arising in a gonadectomized female ce strain mouse has been grown successfully in the following classes of ce mice: (a) gonadectomized females, (b) gonadectomized males, (c) intact females and (d) intact males. It was also grown in two F_1 generation mice where a ce mouse was one of the parents. Evidence was secured that the transplant tumors were associated with an androgenic influence in most instances although under some circumstances there was evidence for an estro-

genic influence. Transplants of adrenal tumor P1699 exerted a restraining effect on the occurrence of primary adrenal cortical tumors.

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Intramedullary Plasma Cell Myeloma Occurring Spontaneously in a Dog

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Some human neoplasms are unknown in animals and there are animal tumors that have not been described in man. Included among the former are new-growths of the myeloma type. Engelbreth-Holm (2) states that plasma cell leukemia and the related multiple myelomatosis has not yet been observed in lower animals. A survey of the veterinary literature discloses no references to intramedullary plasma cell myelomas in any domestic animal. A tumor of this type was recently encountered in a routine necropsy of a dog and this report, therefore, represents the first example of a spontaneous plasma cell myeloma in a lower animal.

MATERIAL AND METHODS

The dog was brought to the animal hospital for euthanasia, which was accomplished with a lethal dose of pentabarbitol given intravenously. Immediate necropsy was performed and the tissues were fixed in Bouin's solution and 10 per cent solution of neutral formaldehyde. Paraffin sections were stained with hematoxylin and eosin, Mallory's phosphotungstic acid hematoxylin, Unna's polychrome methylene blue, methyl green pyronin, Hitchcock and Ehrings mixture, Dominici's and Masson's stains. Frozen sections of formaldehyde-preserved tissue were stained with Sudan IV and Nile blue sulphate.

CLINICAL DATA

The animal was a 12 year old, male English setter that had shown pain and lameness of increasing severity of the left front leg for a period of 18 months. Palpation revealed a painful enlargement of the upper third of the left humerus. The affected limb was held in the characteristic position of radial paralysis with inability to extend the lower portion of the leg. Physical examination was otherwise negative and the owner stated that the dog appeared normal with the exception of the lameness.

RADIOGRAPHIC OBSERVATIONS [1]

Films were made in the antero-posterior and lateral positions after removal of the left humerus from the dog's body and fixation in 10 per cent formalin. As shown in Fig. 1 the bone had been transected just below the head and the two parts separated about 1 cm. The bone structure of the upper two-thirds of the shaft and the upper extremity was definitely abnormal. The cortex particularly of the shaft was considerably thinner than usual. The medullary and subcortical trabecular markings were definitely coarsened and particularly in the mid-third of the shaft of the bone there were inter-trabecular depressions on the inner aspect of the cortex which extended deep into the cortex appearing, in some instances, almost through the cortex. In the upper third of the shaft the breadth of the bone was increased, particularly on the medial aspect of the cortex, and the compact bone of the cortex had entirely disappeared in this region. No complete perforation of the cortex was demonstrated in these films.

In the head and tuberosities the coarsened trabecular structures and thinning of the cortex was as in the upper portion of the shaft of the humerus. There was some periosteal proliferation on the anterior aspect of the upper third of the shaft of the bone.

These x-ray findings indicated the presence of an

[1] By A. L. L. Bell, M.D., Dept. of Radiology, Long Island College of Medicine.

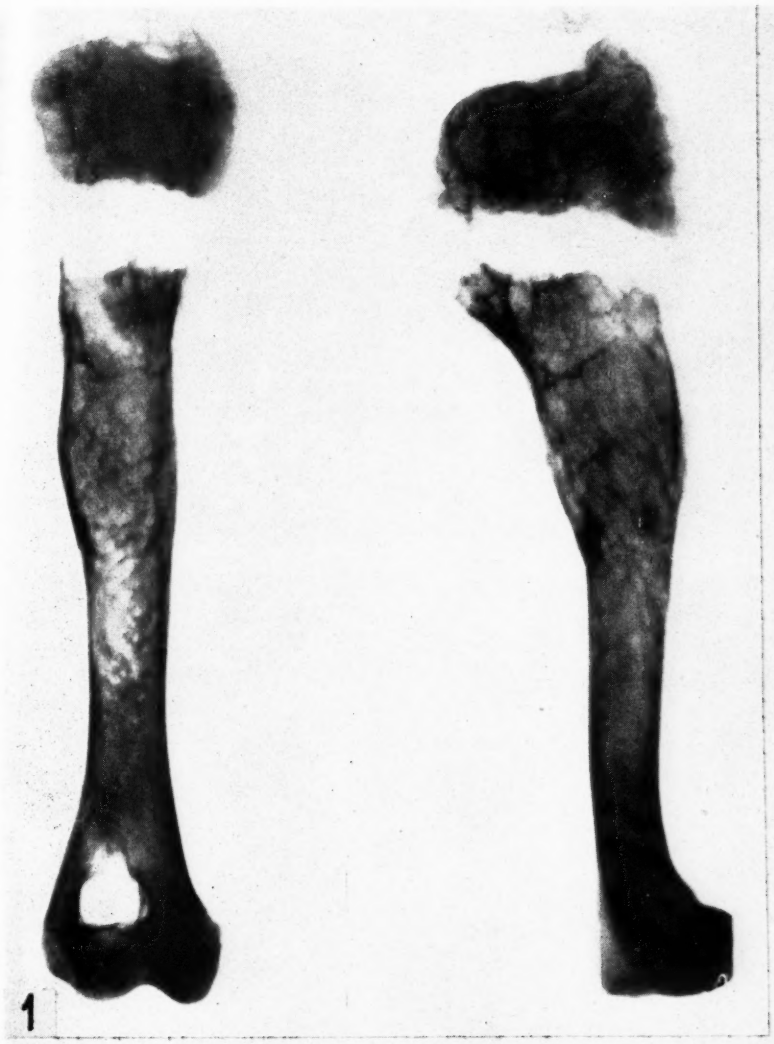
DESCRIPTION OF FIGURES 1 TO 4

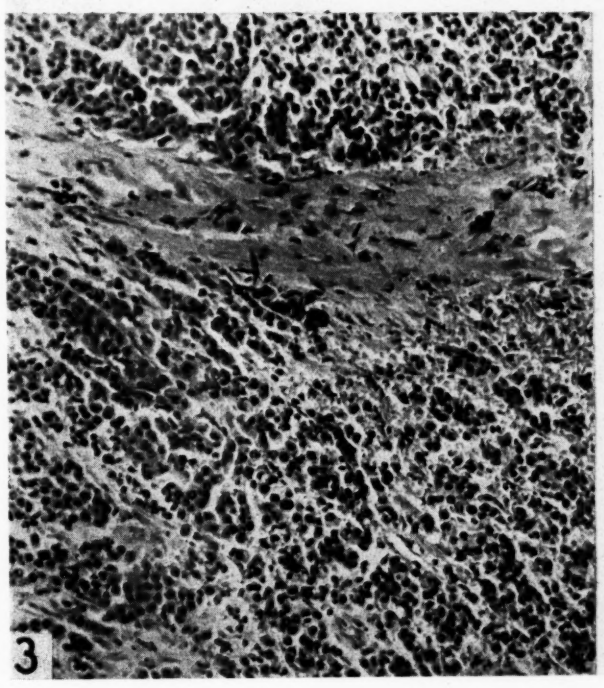
FIG. 1.—Radiographic appearance of plasma cell myeloma involving the upper two-thirds of left humerus.

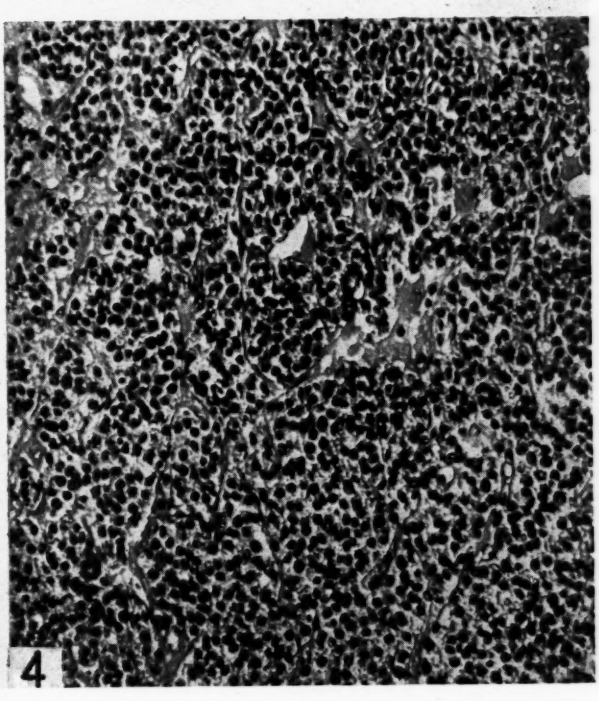
FIG. 2.—Longitudinal section of left humerus illustrating the gross appearance of the neoplasm.

FIG. 3.—Photomicrograph depicting the general tumor structure. A thickened fibrous trabecula is present in the upper portion and the deep-staining granular material is hemosiderin. Dominici's strain. Mag. × 200.

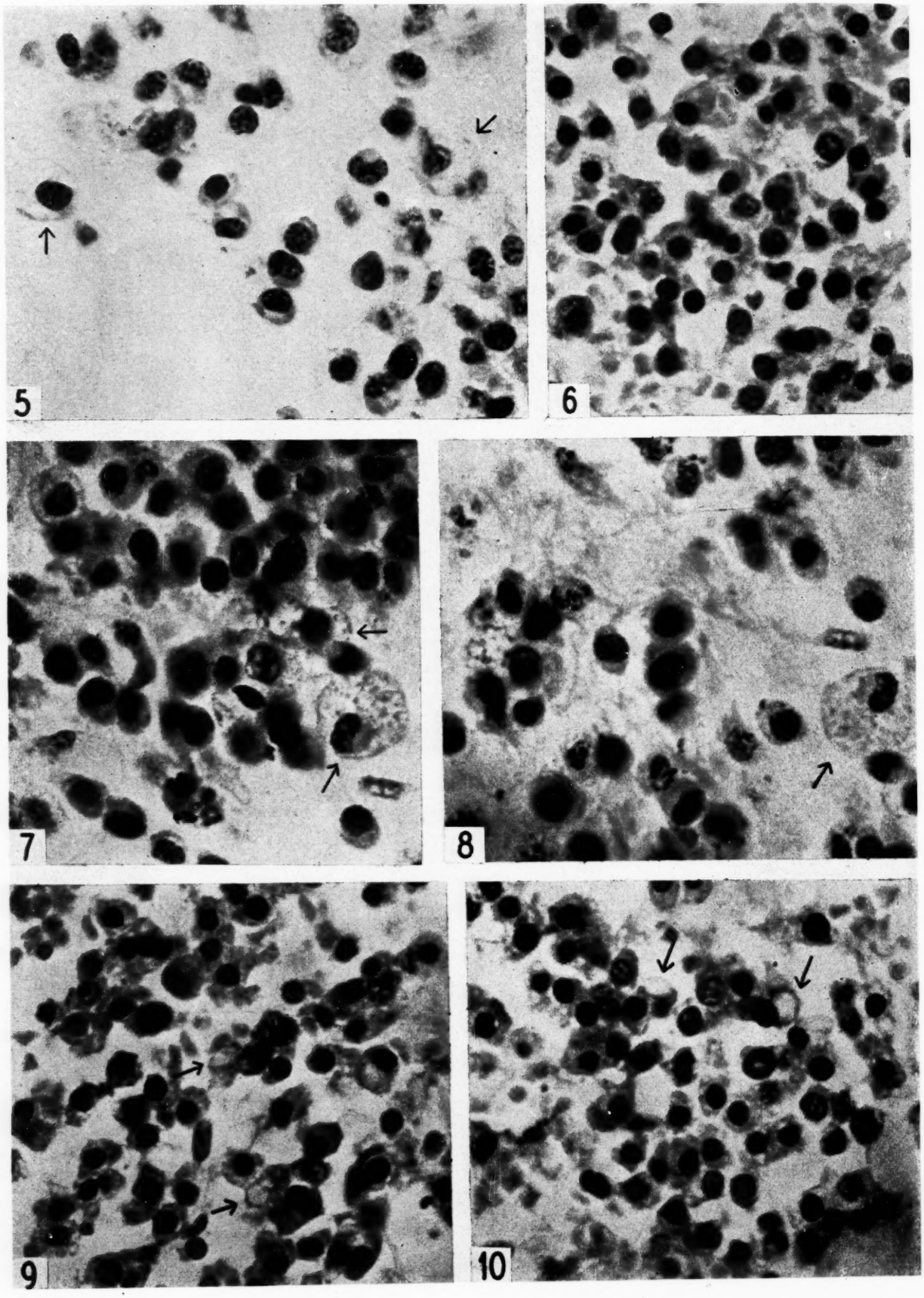
FIG. 4.—Photomicrograph showing the fibrous stroma. Masson's stain. Mag. × 200.







FIGS. 1-4



FIGS. 5-10

expanding tumor originating in the medullary cavity. A diagnosis of malignant growth could not be made on the basis of the x-ray findings alone.

MACROSCOPIC OBSERVATIONS

The entire upper third of the humerus was expanded in thickness and measured 5 cm. in diameter at its widest point (Fig. 2). The periosteum was irregularly thickened and the adjacent muscles were edematous with occasional small hemorrhagic areas. The cortex was thinned and the affected bone cut easily despite the presence of numerous small bony trabeculae. On section, the upper two-thirds of the marrow cavity was replaced with a soft, reddish-grey tissue interspersed with hemorrhagic areas of a deeper red color. Small foci of necrosis and fibrous trabeculae were present. The medullary cavity of the lower third of the bone contained a pale grey, fatty marrow.

MICROSCOPIC OBSERVATIONS

Humerus.—The general cellular structure of the neoplastic cells was similar to that of the plasma cells described in human myelomas (Figs. 5 to 10). The tumor cells were round, oval or polyhedral and the spherical or oval nucleus was centrally or eccentrically located. The nuclear structure consisted of coarse chromatin blocks that were usually marginated on a distinct nuclear membrane and nucleoli were absent. Occasional nuclei were larger and hyperchromatic. Some cells contained two or more nuclei and mitotic figures were not uncommon. The cellular dimensions averaged 9.36 μ and the nuclei averaged 5.39 μ.

The cytoplasm was amphophilic with hematoxylin and eosin, pale brownish-red with Mallory's phosphotungstic acid hematoxylin, pale blue with Dominici, reddish-purple with Masson's stain, pale greenish-blue with Unna's polychrome methylene blue, pale red with methyl green pyronin and pale blue with Hitchcock and Ehring's stain.

In the majority of cells, the cytoplasm was homogeneous, agranular and sometimes contained a paranuclear clear zone. Cytoplasmic vacuoles occurred in occasional cells and fat stains indicated the absence of lipids in the vacuoles (Figs. 9, 10). Scattered among the tumor cells were larger cells that averaged 12.16 μ and whose cytoplasm contained fine or coarse, acidophilic granular structures (Figs. 5, 7, 8). Their nuclei, however, were identical morphologically with those of the myeloma cells. The granular material differed from the granules of eosinophils and existed as irregular clumps and granules. The cytoplasm was frequently vacuolated and the oxyphilic material occurred between the vacuoles and occasionally within the vacuoles. Quantitative variations indicated few granules in some cells and many in others. The granular material stained blue with Mallory's phosphotungstic acid hematoxylin and red with eosin, Dominici and Masson's stain. This granular material did not show special characteristics with Hitchcock and Ehring's stain, polychrome methylene blue and methyl green pyronin.

The tumor structure consisted of large compact masses of cells in loose and solid arrangements, smaller collections and as separated solitary cells (Figs. 3, 4). In certain regions there was considerable fibrosis with hyalinized strands of fibrous connective tissue separating groups of cells so that a somewhat alveolar appearance was produced. Silver stains showed no intercellular reticulum but fine argyrophilic fibers encircled small and large cellular masses. Bony trabeculae in various stages of atrophy and resorption occurred in some regions and foreign body giant cells and fibrosis were not uncommon in their vicinity. The persisting Haversian canals often contained tumor cells. Hemorrhages were extensive in many areas and foci of necrosis were not infrequent. The vascular supply was not conspicuous and golden yellow pigment (hemosiderin) occurred in many localities. The periosteum was thickened and edematous and the neighboring muscles showed edema and atrophic changes. Tumor cells only occasionally invaded the periosteum and muscles. In the tumor tissue, marrow fat persisted in some areas while other regions were completely replaced with neoplastic cells. No marrow cells were seen except rare polymorphonuclear leukocytes, normoblasts and lymphocytes.

Sections of the marrow from the radiographically normal distal portion of the humerus indicated that the cellular components consisted principally of tumor cells. In addition, the sinusoids were often congested and hemorrhagic.

DESCRIPTION OF FIGURES 5 to 10

FIG. 5.—A less compactly grouped collection of tumor cells indicating the resemblance to plasma cells. Several cells have a distinct paranuclear clear zone. The arrows point to larger cells with eosinophilic granular material in the cytoplasm. Dominici's stain. Mag. × 850.

FIG. 6.—Another area showing the cellular structure. Dominici's stain. Mag. × 850.

FIGS. 7, 8.—The arrows indicate larger tumor cells with eosinophilic cytoplasmic granulations. Dominici's stain. Mag. × 850.

FIGS. 9, 10.—Neoplastic cells with cytoplasmic vacuoles (arrows). Dominici's stain. Mag. × 850.

Kidneys.—The epithelium of the proximal portion of the proximal convoluted tubules showed various degrees of degeneration progressing from cloudy swelling to necrosis. Many tubular lumina contained a granular, eosinophilic material. The epithelium of the broad ascending limbs and the distal convoluted tubules manifested these regressive changes to a lesser degree. A conspicuous finding was the presence of giant-sized, hyperchromatic, solitary nuclei with large nucleoli that indicated regenerative activity. These nuclei were often embedded in a deeply eosinophilic cytoplasmic syncytium and showed loss of cellular polarity. A moderate number of collecting tubules contained eosinophilic, hyaline casts in their lumina but no foreign body giant cells encircled them. The glomeruli and arteries were normal and inflammatory cells were absent in the interstitial tissue.

Other organs.—With the exception of nodular fibrous intimal aortic plaques, moderate fatty metamorphosis and focal congestion in the liver, pulmonary anthracosis and physiologic splenomegaly, the remaining organs were normal. Metastatic myeloma lesions were absent in all organs examined.

DISCUSSION

The absence of reports on plasma cell myelomas in animals suggests that these neoplasms are either extremely rare or that they are not recognized. Inasmuch as the described case is the first in a series of over 300 neoplasms observed in a group of 12,000 dogs, the former conclusion seems more plausible.

The microscopic appearance of the dog tumor resembled in all respects the usual myelomas in man. The material at hand offered no solution to the controversy concerning the histogenesis of the myeloma cells. A prominent feature of the canine myeloma was the relatively large number of cells with vacuoles and eosinophilic granular material in the cytoplasm. Similar degenerative forms occur in human myelomas and Michels (4) mentions the presence of vacuoles and granules in inflammatory plasma cells. Miller (5) observed comparable cytoplasmic changes in plasma cells experimentally produced in rabbit omentum by intraperitoneal injections of tuberculoprotein.

The x-ray films resembled those described by Paul and Pohle (6) for human solitary myelomas of bone. The lesions in the dog are similar to solitary myeloma lesions characterized by an osteolytic, multicystic area of rarefaction centrally located and expansive with irregular trabeculae. In the differential roentgen diagnosis, single myelomas can easily be confused with giant cell tumor, localized fibrocystic disease of the bone, osteogenic sarcoma, Ewing's tumor and some of the rarer bone tumors.

The question whether the canine tumor was solitary or multiple cannot be answered with any degree of certainty. Unfortunately, the character of the bone lesion was not suspected until the microscopic slides were studied so that roentgen and histologic examinations of the other bones were not made. That the process was disseminated might be suggested by the microscopic finding of myeloma cells in the radiographically normal distal third of the humerus. The roentgenographic appearance of the upper humerus, however, resembled that of human solitary myelomas.

The renal changes consisting of degenerative and reparative epithelial alterations with occasional casts in the collecting tubules are not the typical findings observed in human myelomas associated with Bence-Jones proteinuria. In the latter, Bell (1), Forbus and his group (3) and others consider that the tubular casts mechanically obstruct the tubules causing tubular dilatation and atrophy. The kidney damage is therefore indirect and is not the result of any specific toxic effect on the tubular epithelium. In the dog case, as no urine tests for Bence-Jones protein were performed, the renal findings preclude any opinions relative to the character of the microscopic kidney changes.

The age and sex incidence of the myeloma in the dog coincided with that observed most commonly in man. In the latter, the disease occurs in later life and males are more frequently affected.

SUMMARY

A review of the literature indicates that myelomas of the plasma cell type are unknown in animals. This report is the first record of such a neoplasm that involved the left humerus of a 12 year old, male dog. The lesion was intramedullary and consisted of cells of the plasma cell type. The histologic structure of the canine myeloma was similar to that seen in human myelomas. The radiographic appearance resembled that of solitary myelomas in man.

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Abstracts

Reports of Research

Observations on the Carcinogenicity of 1,2,3,4-Dibenzophenanthrene and Its 9-Methyl and 10-Methyl Derivatives. HARRIS, P. N., and BRADSHER, C. K. [Duke Univ., Durham, N. C.] *Cancer Research,* **6**:671-673. 1946.

Five mgm. doses of 1,2,3,4-dibenzophenanthrene suspended in tricapryllin or in the ethyl ester of sesame oil were injected subcutaneously into 26 New Buffalo mice. Fifteen developed sarcoma at the site of inoculation after latent periods of 10 to 26 weeks, and 4 others developed epidermoid carcinoma or both epidermoid carcinoma and sarcoma after a lapse of 25 to 42 weeks. The carcinomas appeared only in mice that had developed ulcers at the site of inoculation. Subcutaneous administration of the 9-methyl and 10-methyl derivatives of 1,2,3,4-dibenzophenanthrene did not result in development of tumors.—Authors' abstract.

Carcinogenic Substances in Human Tissues. HIEGER, I. [Royal Cancer Hosp., London, England] *Cancer Research,* **6**:657-667. 1946.

Attention is drawn to the main difficulties in investigations of this type. Apparently human tissue carcinogens are of low potency as measured in mice since the latent periods for tumor production are so prolonged. These substances are sporadic in their occurrence as pointed out by Steiner, and there is always uncertainty with regard to "susceptibility" of the experimental mice. A technic of fractionation of unsaponifiable material from human tissues is described, and an active fraction producing sarcomas in mice has been obtained from mixed lung-kidney-muscle tissue of cancerous and non-cancerous human subjects, and also from the liver of a cancerous patient. In all cases the carcinogenic substance is found in the cholesterol-rich fraction of the unsaponifiable material. This fraction is a crystalline mixture of compounds containing on the average about 85% cholesterol.—E. W. S.

Tumors of the Salivary and Parathyroid Glands in Rats Fed with 2-Acetylaminofluorene. HEIMAN, J., and MEISEL, D. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] *Cancer Research,* **6**:617-619. 1946.

Thirty-six female and 23 male Wistar rats 2½ to 3 months old were fed an oily mixture of 2-acetylaminofluorene by syringe and curved needle introduced into the pharynx. The total amount fed ranged between 250 mgm. and 617.5 mgm. during 111 to 227 days. Twenty-two of the treated rats developed nodular swellings of the neck, distributed to 1 or both submaxillary regions, and extending to the subcutaneous areas of the chest and axillae. Autopsy revealed enlarged submaxillary nodes and many small discrete or large confluent cysts. Twelve animals developed adenocarcinoma and adenoma of the submaxillary gland, sarcoma of the subcutaneous tissue of the neck, mammary adenocarcinoma, and adenoma of the liver and parathyroid. The localization of cysts and tumors in the neck may be attributed to trauma of the pharynx and esophagus produced by the needle. The 2-acetylaminofluorene may therefore exert a local action if applied frequently to the same site.—Authors' abstract.

Localization of Stratum of Maximum Mitotic Frequency in Epidermal Methylcholanthrene Carcinogenesis in Mice. COWDRY, E. V., VAN DYKE, J. H., and GEREN, B. B. [Washington Univ., and Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] *Cancer Research,* **6**:620-624. 1946.

Of 5 male New Buffalo mice, 6 weeks old, the backs of which had been treated 3 times with 0.6% methylcholanthrene in benzene, 4 showed a greater mitotic frequency in the basal epidermal layer than in any of the rows of spinous cells, while 1 showed a greater frequency in the basal layer than in the first row of spinous cells and less than in the second row 10 days after the initial treatment. Five similar mice showed maximum mitotic frequency in the basal layer 20 days after the first application of carcinogen. Though considerable variability was observed in individual ratios of mitoses to nondividing nuclei, the conclusion is advanced that under the conditions of the experiment maximum mitotic frequency is predominately in the single layer of basal cells in touch with the basement membranes.—Authors' abstract.

The Inhibition of the Carcinogenicity of *p*-Dimethylaminoazobenzene by Certain Detergents and the Effect of Diet on the Levels of Azo Dyes in Rat Tissues. MILLER, J. A., KLINE, B. E., and RUSCH, H. P. [Univ. of Wisconsin Med. Sch., Madison, Wis.] *Cancer Research,* **6**:674-678. 1946.

Seven groups of 15 rats each were fed 0.06% of *p*-dimethylaminoazobenzene for 4 months in synthetic diets containing: 5% of corn oil; or 5% of corn oil plus 0.25% of either of 2 commercial synthetic detergents, Tergitol Penetrants 4 or 7; or 5% of mineral oil. The dye-free diets were then fed for 2 more months. In the three control groups fed the diet containing 5% of corn oil the final incidences of liver tumors were 93, 63, and 80%. No tumors occurred when the diets contained either detergent or when the corn oil was replaced by mineral oil. These substances represent new types of dietary inhibitors for *p*-dimethylaminoazobenzene in the rat.

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Analyses were made for the 3 basic azo dyes in the liver and for *p*-aminoazobenzene in the blood of rats fed *p*-dimethylaminoazobenzene in various diets known to affect the activity of this dye. Neither protective nor stimulatory diets produced any large changes in the concentrations of the dyes in these tissues. These data provide no conclusive support for the possibility that the detergents, mineral oil, and other inhibitors interfere with the absorption or transport of the dye.—Authors' summary.

The Metabolism of 3,4-Benzpyrene into 8- and 10-Benzpyrenols in the Animal Body. BERENBLUM, I., and SCHOENTAL, R. [Univ. of Oxford, Oxford, England]—**With an Appendix on Absorption Spectra.** HOLIDAY, E. R., and JOPE, E. M. [London Hosp., London, England] *Cancer Research,* **6**:699-706. 1946.

After intraperitoneal injection of 3,4-benzpyrene into rabbits, the feces were found to contain 8- and 10-benzpyrenols and 5,8- and 5,10-benzpyrenequinones.

A reinvestigation of the metabolism of benzpyrene in rats revealed the presence of the same products in their feces, though the relative amounts of 10-benzpyrenol and 5,10-benzpyrenequinone in relation to the 8- and 5,8-isomers, were less in the rat than in the rabbit extracts.

Evidence was obtained of the presence in rabbit and rat feces of other fluorescent products, probably derived from benzpyrene. Their nature has not yet been determined.—Authors' summary.

Effect of Progesterone on the Development of Mammary Cancer in C3H Mice. BURROWS, H., and HOCH-LIGETI, C. [Royal Cancer Hosp., London, England] *Cancer Research,* **6**:608-609. 1946.

Thirty mice of a high mammary cancer strain (C3H) received subcutaneous injections of 1 mgm. of progesterone weekly. No differences could be established in the frequency, and the time of the appearance of tumors in these mice and in the 20 control animals receiving injections of the solvent only. During the first 3 to 4 weeks of the experiment 2 of the treated and 1 control animal died without tumors.—Authors' summary.

The Influence of Sex Hormones upon the Hepatic Lesions Produced by 2-Acetaminofluorene. CANTAROW, A., PASCHKIS, K. E., STASNEY, J., and ROTHENBERG, M. S. [Jefferson Med. Coll., Philadelphia, Pa.] *Cancer Research,* **6**: 610-616. 1946.

Excessive quantities of endogenous or exogenous estrogen and androgen accelerated and intensified the development of cystic and neoplastic hepatic lesions induced in Sherman rats by 2-acetaminofluorene. This phenomenon may be related to the role of the liver in the intermediary metabolism and excretion of the sex steroids. Thiouracil appeared to exert a strikingly protective action upon the liver as regards the development of both cystic and neoplastic lesions. No tumors occurred in the hyperplastic "target organs" of the sex hormones, in sharp contrast to the high incidence of tumors of the thyroid in rats receiving thiouracil simultaneously with the carcinogen. The fact that the hyperplasia induced by the sex hormones is a functioning hyperplasia whereas that induced in the thyroid by thiouracil is functionless, may possibly be a determining factor in the relationship between simple and neoplastic growth of tissue.—Authors' abstract.

Neoplasms of the Adrenal Cortex in Noncastrate Mice. KIRSCHBAUM, A., FRANTZ, M., and WILLIAMS, W. L. [Univ. of Minnesota Med. Sch., Minneapolis, Minn.] *Cancer Research,* **6**:707-711. 1946.

In the NH stock of mice gonadectomy is not necessary to induce the development of cortical neoplasms of the adrenal gland. Adenomas of the adrenal cortex appeared spontaneously in 13 of 14 intact female mice over 1 year of age. In 1 instance the secretion of relatively large amounts of estrogenic hormone by such an adenoma was demonstrated. Cortical adenomas are probably relatively infrequent in male mice of this stock. Two histologically malignant tumors found in males are described. The testes of both mice were spermatogenically active.—Authors' abstract.

Induction of Neoplasia *in Vitro* with a Virus. Experiments with Rabbit Skin Grown in Tissue Culture and Treated with Shope Papilloma Virus. COMAN, D. R. [Univ. of Pennsylvania Sch. of Med., Philadelphia, Pa.] *Cancer Research,* **6**:602-607. 1946.

Experiments were designed to determine whether neoplasia can be induced in tissue cultures of rabbit skin by papilloma virus.

The criteria adopted for induction of neoplasia were: (a) Increased growth activity of epithelial cells in the cultures after introducing papilloma virus. (b) Formation of relatively large tumors in the liver of the rabbit following implantation of such tissue cultures. Both criteria were met by the experiments and it is therefore concluded that papilloma virus is capable of inducing neoplasia *in vitro,* namely in tissue cultures of rabbit skin.—Author's abstract.

Stimulation and Retardation of Neoplastic Growth by Sulfhydryl Compounds. BRUNSCHWIG, A., ARNOLD, J., and EDGCOMB, J. [Univ. of Chicago, Chicago, Ill.] *Cancer Research,* **6**:560-562. 1946.

The injection of certain —SH compounds (thioglycollate and thiomalate), which afford available —SH radicals, showed mild stimulation of growth of the transplantable rat tumor 256. The explanations of failure of other —SH compounds (methionine, cysteine, thiolactate) to cause such stimulation are discussed. The injection of —SH inhibitors, iodoacetate and maleate, was followed by retardation of growth of rat tumor 256 but did not inhibit the establishment of the transplants.—Authors' summary.

Intracellular Distribution of Enzymes. II. The Distribution of Succinic Dehydrogenase, Cytochrome Oxidase, Adenosinetriphosphatase, and Phosphorus Compounds in Normal Rat Liver and in Rat Hepatomas. SCHNEIDER, W. C. [Med. Sch., Univ. of Wisconsin, Madison, Wis.] *Cancer Research,* **6**:685-690. 1946.

Normal rat liver and rat hepatomas were separated by centrifugation into a nuclear fraction, a large granule fraction, and crude residue. Succinic dehydrogenase, cytochrome oxidase, adenosinetriphosphatase, pentose and desoxypentose nucleic acids, acid soluble and lipid phosphorus, "protein" phosphorus and nitrogen, and dry material were determined in the original tissue homogenate and on each of the tissue fractions. The activities of succinic dehydrogenase and cytochrome oxidase were much lower in the hepatoma than in the normal liver. However the major part of the enzyme activities associated with the

original tissues was found to be associated with the large granule fractions of these tissues, and the enzyme activities per unit of dry material or of "protein" nitrogen were similar in the large granule fraction of the 2 tissues. Thus the decreased enzyme activity observed in the hepatoma seemed to be due at least in part to a decrease in the amount of large granule material. The activity of adenosinetriphosphatase was essentially the same in the hepatoma as in the normal liver although the distribution of the enzyme was profoundly different in the two tissues. In the latter about 50% of the activity was associated with the large granules and 30% with the unfractionated residue while in the case of the hepatoma 75% of the enzyme activity was associated with the unfractionated residue and only 12% with the large granules. The enzyme activity per unit of dry weight or of "protein" nitrogen was similar for both tissues in the large granule fraction. The desoxypentose nucleic acid content of the hepatoma was found to be more than twice as great as the content of normal liver. All of the desoxypentose nucleic acid present in the whole homogenates was recovered in the nuclear fractions. The increased content of this nucleic acid in the hepatoma was considered to be due to an increase in the number of cells in this tissue. The pentose nucleic acid content of the 2 tissues was found to be essentially the same and the major part of this nucleic acid was found in the unfractionated residue. The nucleic acid content per mgm. of dry material was higher in the large granule fraction and in the unfractionated residue of the hepatoma than in normal liver. The major portions of the other components measured were found in the unfractionated residue. The lipid phosphorus was more concentrated in the large granule fractions of the 2 tissues and more concentrated in the cytoplasm fractions of the hepatoma than in the corresponding fractions of normal liver.—Author's abstract.

Demonstration of an Enzyme-Inhibiting Factor in the Serum of Cancer Patients. (A Preliminary Study). Hirshfeld, S., Duboff, G., and West, P. M. [Cedars of Lebanon Hosp., Los Angeles, Calif.] *Cancer Research,* **6**:57-60. 1946.

Utilizing the aerobic oxidation of tyrosine in the presence of tyrosinase and light, which develops a color complex from the colorless monohydrated phenolic tyrosine to the pink dioxyquinone, a method is described for the estimation of the rate of activity of the tyrosinase by a quantitative estimation of the color complex produced in the reaction by the use of a photoelectric colorimeter. This work is presented as a new approach to the field of cancer enzymology.—Authors' summary.

Phosphorus Compounds in Animal Tissues. IV. The Distribution of Nucleic Acids and Other Phosphorus-Containing Compounds in Normal and Malignant Tissues. Schneider, W. C., and Klug, H. L. [Med. Sch., Univ. of Wisconsin, Madison, Wis.] *Cancer Research,* **6**:691-694. 1946.

The desoxypentose nucleic acid, pentose nucleic acid, acid soluble phosphorus, lipid phosphorus, nucleic acid phosphorus, and "protein" phosphorus contents of several normal rat tissues and of several rat and mouse tumors were determined. It was found that the desoxypentose nucleic acid and the pentose nucleic acid contents were relatively constant in the different neoplasms, while the nucleic acid contents of the normal cells varied considerably in the different tissues. The acid soluble, lipid, and "protein" phosphorus contents of the neoplastic tissues fell within the range covered by the normal tissues. A study of the individual compounds which comprise these fractions will be necessary to decide whether any marked differences exist between these fractions in the normal tissues and in the neoplastic tissues.—Authors' abstract.

The Effect of Certain Diets on Hepatic Tumor Formation Due to *m'*-Methyl-*p*-Dimethylaminoazobenzene and *o'*-Methyl-*p*-Dimethylaminoazobenzene. Giese, J. E., Clayton, C. C., Miller, E. C., and Baumann, C. A. [Univ. of Wisconsin, Madison, Wis.] *Cancer Research,* **6**:679-684. 1946.

m'-Methyl-*p*-dimethylaminoazobenzene was fed to rats in 4 different rations known to affect the formation of tumors due to *p*-dimethylaminoazobenzene. The *m'* methyl dye was fed at several concentrations and for different periods of time.

In general diet was relatively ineffective in altering the rate of tumor development due to *m'*-methyl-*p*-dimethylaminoazobenzene. Hydrogenated coconut oil did not exert a consistent protective effect, while rice bran extract stimulated tumor formation only slightly.

Riboflavin usually retarded the formation of tumors due to the *m'*-methyl dye, but the effect of the vitamin was less than that previously observed when *p*-dimethylaminoazobenzene was the carcinogen.

Rice bran concentrate stimulated tumor formation due to *o'*-methyl-*p*-dimethylaminoazobenzene whereas either hydrogenated coconut oil or riboflavin retarded it. The effects of diet against this carcinogen were intermediate between those observed against *p*-dimethylaminoazobenzene and *m'*-methyl-*p*-dimethylaminoazobenzene.

The variable effects of diet against the different azo dyes suggest that riboflavin may retard tumor formation by interfering with the essential carcinogenic reaction, but that the other diets studied more probably exert their effects upon the carcinogen.—Authors' abstract.

Multiple Peritoneal Sarcoma in Rats from Intraperitoneal Injection of Washed, Ground *Taenia* Larvae. Dunning, W. F., and Curtis, M. R. [Detroit Inst. of Cancer Research, Wayne Univ. Coll. of Med., Detroit, Mich.] *Cancer Research,* **6**:668-670. 1946.

Living parasites were removed from uninvolved cysts of rats with and without gross *Cysticercus* tumors, washed in large volumes of sterile saline, cut in fragments, ground in a motar and suspended in saline. The saline suspension was injected into the peritoneal cavity of uninfested rats which were related to the original hosts of the parasites, and also inoculated in a similar manner into unrelated rats. Multiple peritoneal sarcomas and mesotheliomas were observed within 23 to 787 days following injection. The related rats responded more quickly and more frequently than the unrelated rats.

The agent appears to be more effective in parasites obtained from uninvolved cysts of hosts bearing induced *Cysticercus* sarcoma. Among 56 related rats injected with

the larvae suspension obtained from rats bearing *Cysticercus* sarcoma 51 or 91% died with multiple peritoneal sarcomas in an average of 89 days. When the larvae suspension was obtained from rats with no gross tumor only 22 or 63% of the 35 related rats developed peritoneal sarcomas and the average latent period was 286 days. There is some evidence that the active agent may be associated with the calcium carbonate corpuscles normally present in the coelomic cavity of the parasites.—Authors' abstract.

Further Studies of Specific Precipitin Antiserums for the Protein of Cancer Tissue. III. Relation of the Proteins of Different Malignant Tissues to Each Other. MANN, L. S., and WELKER, W. H. [Univ. of Illinois Coll. of Med., Chicago, Ill.] *Cancer Research,* **6**:625-626. 1946.

Antisera for carcinoma of the stomach, breast, kidney, rectum, and metastatic carcinoma of the liver were precipitin-tested against autolysates of carcinomas of the breast, sarcoma of the breast, carcinomas of the cervix, colon, and kidney, hypernephromas, metastatic carcinomas of the liver, and carcinomas of the ovary, stomach, and urinary bladder as well as of the rectum. Some antisera showed interreaction with nonhomologous autolysates whereas antiserum for carcinoma of 1 breast specimen was relatively specific, as it reacted only with its own autolysate and autolysates of one carcinoma of ovary. Some of the antisera reacted only with their homologous autolysates. The protein prepared by malignant cells may be dependent on its environment and forces to which it is subjected. The malignant cells may have lost the ability of manufacturing a specific protein, characteristic of itself. There are probably a wide variety of proteins manufactured by malignant cells.—Authors' abstract.

Attempted Transmission of Acute Leukemia from Man to Man by the Sternal Marrow Route. THIERSCH, J. B. [Inst. of Med. and Veterinary Science, Adelaide, Australia] *Cancer Research* **6**:695-698. 1946.

Attempts were made to transmit acute leukemia from man to man. Bone marrow from four cases of leukemia was implanted in the sternal marrow of 12 patients, previously treated for carcinoma of the tongue. No leukemic reactions developed in the recipients after the implantations during a period of observation of 101 days to more than two years.—Author's abstract.

On the Transplantability of Lymphoid Tumors, Embryonal Nephromas and Carcinomas of Chickens. DURAN-REYNALS, F. [Yale Univ. Sch. of Med., New Haven, Conn.] *Cancer Research,* **6**:545-552. 1946.

The transplantation of 12 lymphoid tumors under the same conditions that have proved successful for some chicken sarcomas failed entirely. Likewise 2 epithelial growths could not be transplanted. Ten embryonal nephromas are described; 1 of them was carried through a single chicken passage where it grew in a manner of a sarcoma in one bird. Another, probably a fibroma, was transplanted. The sarcomatous growths accompanying a third case were transplanted indefinitely as sarcomas, and a causative virus was demonstrated even in the original growths. Since unmistakable metastases of the embryonal nephroma occurred in at least 3 cases and in one of them there were also present sarcomatous growths we must consider the possibility that the transplantable infectious sarcomas may have been metastases of the embryonal nephroma. The factors that seem to govern the transplantability of all spontaneous chicken tumors are discussed.—Author's abstract.

A Transplantable 9,10-Dimethyl-1,2-Benzanthracene Sarcoma in the Syrian Hamster. CRABB, E. D. [Univ. of Colorado, Boulder, Colo.] *Cancer Research,* **6**:627-636. 1946.

The 311 male and female hamsters used in this work showed 100% takes when 6 lines of tumors, all derived from 1 benzanthracene-induced, mixed-cell sarcoma, were reciprocally transplanted into this species, but transplants to 35 mice did not take. Transplants of growing and necrotic tumors and injections of liquid necrotic material usually resulted in growths in 6 to 10 days after inoculation, but this latent period was commonly increased about 10 days and the percentage of takes reduced about 50% by injecting blood from incised solid tumors. After adding fragments of the tumor to the blood, by scraping the sides of the incision, both latent period and takes were about normal. Prolonging the lives of the hosts by removing the tumors 1 or 2 times increased the incidence of single and of multiple metastases. Tumors induced by benzanthracene, those that had been transplanted several generations and those resulting from grafts of metastatic deep axillary lymph nodes, lungs and kidneys, were equally potent in producing metastases. In two hamsters, intrajejunal injections of the sarcoma resulted in metastasis to a mesenteric lymph node in one, and cancer cell invasion of these nodes in both animals. There was no definite histologic difference in any of the mature tumors or advanced metastases, but transient differences were noted in some of the newly formed metastases.—Author's abstract.

Transplantation of an Adrenal Cortical Carcinoma. WOOLLEY, G. W., and LITTLE, C. C. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Maine] *Cancer Research,* **6**:712-717. 1946.

An adrenal cortical tumor, P1699, arising in a gonadectomized female ce strain mouse has been grown successfully in the following classes of ce mice: (a) gonadectomized females, (b) gonadectomized males, (c) intact females and (d) intact males. It was also grown in two F_1 generation mice where a ce mouse was one of the parents. Evidence was secured that the transplant tumors were associated with an androgenic influence in most instances although under some circumstances there was evidence for an estrogenic influence. Transplants of adrenal tumor P1699 exerted a restraining effect on the occurrence of primary adrenal cortical tumors.—Authors' abstract.

A Comparative Morphological Study of the Mammary Glands with Reference to the Known Factors Influencing the Development of Mammary Carcinoma in Mice. HUSEBY, R. A., and BITTNER, J. J. [Univ. of Minnesota, Minneapolis, Minn.] *Cancer Research,* **6**:240-255. 1946.

A histological study was made to correlate the architecture of the mouse mammary gland and the 3 "primary" factors required for spontaneous mammary carcinogenesis: an inherited susceptibility, quantitatively and/or qualita-

tively adequate hormonal stimulation, and the milk influence. For this investigation several low tumor lines of mice, each lacking a single though different, "primary" factor, were selected and compared with suitable mice that possessed all 3 of these factors and thus had a high incidence of mammary cancer. In this way the effects of a lack of each factor could be determined individually, and the following points were established:

1. The presence of the milk influence *per se* does not alter the extent to which lateral buds occur along the larger ducts of the mammary gland.

2. In the present material, as well as in that previously reported by other authors, lateral budding is more extensive in virgin mice of strains that possess the inherited hormonal influence than in those that lack this factor.

3. Precancerous nodules of alveolar hyperplasia occur frequently only in mice of high tumor groups and are very uncommon in those of low tumor lines, irrespective of which one of the "primary" factors is lacking. From this it is concluded that the same 3 factors that are etiologically important for the development of mammary cancer are necessary for the development of precancerous alveolar hyperplasias.

4. Areas composed of an overgrowth of fine ducts were encountered in the mammae of mice belonging to high tumor lines. These, in all probability, are also precancerous in nature, but because they occur with relative infrequency they cannot represent a very common source of malignant transformation.

5. Inflammatory nodules, consisting of some alveolar hyperplasia usually exhibiting squamous metaplasia of the glandular epithelium and a surrounding inflammatory reaction, did not appear to be precancerous in nature. These occur with equal frequency in low and high tumor strains, and no transitions between them and frank carcinoma could be demonstrated. Etiologic factors important for the development of this type of lesion could not be determined completely, but pregnancy and/or lactation were found to favor their development.—Authors' summary.

The Pathology of Malignant Histiocytoma (Reticuloendothelioma) of the Liver in Mice. GORER, P. A. [Guy's Hosp. Med. Sch., London, England] *Cancer Research,* **6**:470-482. 1946.

Histiocytomas of the liver have been principally studied in the C57 black strain of mice. They occur spontaneously in mice upwards of 18 months old, but may occur considerably earlier in those treated with carcinogenic hydrocarbons. The growths are apparently of multicentric origin, arising in the sinusoids. Extramedullary myelopoiesis, which may accompany them, is generally perivascular in origin. A description of nonmalignant histiocytes is given. Forms intermediate between histiocytes and monocytes may be found, while the nuclear structure may often resemble that of a lymphocyte. Impression smears of the liver show that the dominant cell is a functionally mature histiocyte, and the heart's blood generally contains large numbers of malignant cells. The blood in peripheral circulation shows a variable picture; a few malignant cells probably occur in all late cases, but a true leukemic blood picture is exceptional. Histiocytomas may have the morphology of a spindle cell sarcoma, particularly in the metastases or on transplantation.

Two cases (H.R.1 and H.R.2) have been transplanted. In the earlier transfers both showed a definite tropism for the liver. Both have shown variations in tissue specificities. Once the tropism for the liver was lost they produced a malignant plastic peritonitis with metastases. The peritoneal deposits were spindle-celled. Giant cells occurred occasionally in H.R.1, but more abundantly in H.R.2. H.R.1 now has the characteristics of an acute leukemia. The dominant cell is small and primitive, resembling a lymphoblast. It has retained some power of differentiation. During its period of most rapid growth the cells of H.R.2 showed certain primitive features, particularly in nuclear structure. On 1 occasion during this period the blood of the peripheral circulation showed the picture of a monocytic leukemia.

The question of the terminology of neoplasms of reticuloendothelial origin is discussed. It is concluded that the histiocyte is derived from the primitive reticular cell of Maximov through an intermediary histioblast.—E. W. S.

Tubular Adenomas and Testis-Like Tubules of the Ovaries of Aged Rats. ENGLE, E. T. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] *Cancer Research,* **6**:578-582. 1946.

Three cases of tubular adenomas of the ovaries of aged Wistar rats are described. The characteristic nucleus of the Sertoli cell of the testis is present in these tubular adenomas. In the ovaries of other Wistar rats, in which the cortical ova and follicles have disappeared, are many isolated tubules having the same structural characteristics as the adenomas. The embryological significance of these findings is discussed.—Author's abstract.

Intramedullary Plasma Cell Myeloma Occurring Spontaneously in a Dog. BLOOM, F. [Long Island Coll. of Med., Brooklyn, N. Y.] *Cancer Research,* **6**:718-722. 1946.

Intramedullary plasma cell myelomas are neoplasms that have not been reported in the literature in animals. A tumor fulfilling the morphologic criteria of human plasma cell myeloma was observed in a 12 year old, male English setter. The growth involved the upper two-thirds of the left humerus and was radiographically characterized by a centrally located expansive, multicystic area of rarefaction with irregular trabeculae and thinning of the cortex. Grossly, the proximal two-thirds of the medullary cavity was replaced with a soft, reddish-grey tissue in which small bony trabeculae were present. Microscopically, the tumor cells resembled the plasma cells of human myelomas. In addition, a moderate number of neoplastic cells contained vacuoles or eosinophilic granular material in their cytoplasm. The tumor structure consisted of large collections of plasma cells with considerable fibrosis in regions. Bony trabeculae were common and necrosis and hemorrhage occurred not infrequently. The radiographically normal distal third of the humerus evidenced microscopic replacement of the cellular elements by myeloma cells. Histologic examination of the parenchymal organs disclosed no metastatic myeloma lesions. There was no renal lesion of the sort commonly associated with myelomas in man.—Author's abstract.

The Effect of Nitrogen Mustards on the Viscosity of Thymonucleate. GJESSING, E. C., and CHANUTIN, A. [Univ. of Virginia, Charlottesville, Va.] *Cancer Research,* **6**: 593-598. 1946.

Data are presented to show that the viscosity of thymus nucleate solutions is decreased by nitrogen mustards, particularly when the ionic strength of the solution is low. It has been shown that the ethylene-imine ring transformation product of these mustards appears to be responsible for the depolymerizing effect. Thiosulfate is capable of completely inhibiting the effects of the ethylene-imine ring.—Authors' abstract.

The Effect of Nitrogen Mustards upon the Ultraviolet Absorption Spectrum of Thymonucleate, Uracil and Purines. CHANUTIN, A., and GJESSING, E. C. [Univ. of Virginia, Charlottesville, Va.] *Cancer Research,* **6**: 599-601. 1946.

Spectrophotometric evidence is presented to show that the nitrogen mustard, *tris* (β chloroethyl) amine, reacts with sodium thymonucleate, adenine, guanine, xanthine, and uracil.—Authors' abstract.

Clinical and Pathological Reports

Clinical investigations are sometimes included under Reports of Research

THERAPY—GENERAL

Terminal Care in Cancer. ABRAMS, R., JAMESON, G., POEHLMAN, M., and SNYDER, S. [Massachusetts Gen. Hosp., New England Deaconess Hosp., Boston Dispensary, and Beth Israel Hosp., Boston, Mass.] *New England J. Med.,* **232**:719-724. 1945.

A study by social workers of 200 patients attending Boston clinics, with recommendations as to proper management.—C. W.

FEMALE GENITAL TRACT

Bilateral Brenner Tumors of the Ovaries: Report of a Case. JOHNSON, J. R., and DOCKERTY, M. B. *Proc. Staff Meet., Mayo Clin.,* **20**:120-123. 1945.

The authors present a case in which it is pointed out that Brenner tumor may be mistaken for a primary or metastatic epithelioma. The predominant fibrous nature of the growth, the complete absence of mitosis, and the peculiar combination of islands of squamous cells showing central transitions to columnar elements are the salient points in the diagnosis.—J. L. M.

Case Reports of Barnes Hospital. Clinical and Postmortem Records Used in Weekly Clinicopathologic Conferences at Barnes Hospital, St. Louis. WOOD, W. B., JR., and MOORE, R. A., EDITORS. *J. Missouri M. A.,* **42**:697-708. 1945.

Two cases of cancer are presented. The first was a case of pseudomucinous cystadenocarcinoma involving the left periovarian tissue, peritoneum, liver, lungs, spleen, kidneys, heart, vertebrae, left ureter, left common iliac and peripancreatic lymph nodes. Renal cell carcinoma of the right kidney, with carcinoma in and occluding the right renal vein and inferior vena cava and within the right atrium of the heart, was found in the second case. There was also invasion of the wall of a hepatic vein by carcinoma and metastasis to the upper lobe of the left lung.—M. E. H.

Giant Cystic Arrhenoblastoma of the Ovary Containing Entodermal Epithelium and a Carcinoid. HARTZ, P. H. [Pub. Health Service, Curaçao, Netherlands West Indies] *Am. J. Path.,* **21**:1167-1191. 1945.

A case report.—J. G. K.

Bilateral Ovarian Carcinoma in a Thirty Week Fetus. ZIEGLER, E. E. [St. Luke's Hosp., Bethlehem, Pa.] *Arch. Path.,* **40**:279-282. 1945.

A unique case of carcinoma occurring in fetal life is reported.—J. G. K.

The Treatment of Myomas in Pregnancy Which Are Undergoing Degeneration. JOHNSTON, H. W. [Toronto, Canada] *Canad. M. A. J.,* **53**:366-367. 1945.

Ten cases of myoma undergoing severe degeneration in pregnancy are presented. Treatment should be conservative. Five patients had natural spontaneous deliveries; 5 patients were treated by cesarean section with myomectomy.—M. E. H.

MALE GENITAL TRACT

Choriocarcinoma of the Testicle. GILL, A. J., CALDWELL, G. T., and GOFORTH, J. L. [Southwest. Med. Coll., and St. Paul's Hosp., Dallas, Tex.] *Am. J. M. Sc.,* **210**:745-751. 1945.

The authors conclude, after a histologic study of 3 recent cases and consideration of the literature, that choriocarcinoma of the testicle arises from primitive cells with essentially the same capacity as the developing ovum, and that the malignant trophoblastic elements of this tumor are derived from ectoderm in the same way as the comparable tissue in ordinary pregnancy. They consider that the cells from which the tumor arises are multipotential sex cells, probably spermatogonia, and that tumor cells of this type alone are responsible for the production of gonadotrophic hormones.—J. G. K.

Advanced Cancer of the Prostate. PARLOW, A. L. [Univ. of Rochester Sch. of Med., Rochester, N. Y.] *N. Y. State J. M.,* **45**:383-387. 1945.

During the period between January 1941, and November 1943, 75 cases of advanced cancer of the prostate were treated with orchiectomy. Eighteen of these cases received prostatic resection in addition to castration. The diagnosis, in 66 cases, was proved by microscopic examination. In the remainder of the series the diagnosis was made by rectal examination and by x-ray studies. All of these patients complained of symptoms referable to the act of urination, varying from frequency to complete retention. Forty-two patients complained of low-back pain and loss of weight. Hematuria was a symptom in only 7 patients. In 23 cases there was no clinical or x-ray evidence of any metastasis. Radical perineal prostatectomy was considered inadvisable, inasmuch as the disease was not confined within the capsule of the prostate, in all of the cases.

Eighteen patients were found to have a normal serum phosphatase estimation prior to orchiectomy. In the remainder of the series there was an elevation of the serum phosphatase, and one was recorded at 155 units, using the King-Armstrong technic.

Following orchiectomy 18 patients were considered as complete clinical failures. In no instance was there any relief of pain and the progressive advancement of the disease was in no way altered. Stilbestrol was administered to these patients and found to be effective. In this group microscopic examination of tissue from the prostate revealed carcinoma of the undifferentiated type. Fifty-seven patients showed immediate clinical results characterized by loss of all metastatic pain and general subjective improvement; 43 of this group must be considered as delayed failures, inasmuch as all again developed symptoms of advanced carcinoma of the prostate after intervals of from 8 to 30 months. Examination revealed only partial regression of the disease in 21 of these cases. The microscopic examination of tissue from the prostates in this group revealed no definite histologic arrangement of the carcinoma. Areas of both the adenocarcinoma and undifferentiated type of carcinoma were the characteristic findings.

Of interest is the fact that following orchiectomy 36 patients had complete clinical disappearance of the carcinoma. However, of this number only 14 were symptom-free and showed no evidence of any return of the disease after an interval of 12 to 29 months. It is noteworthy that complete regression of the cancer occurred only in those cases in which microscopic examination of tissue from the prostate revealed a typical adenocarcinoma. Serum phosphatase estimations were obtained for all patients following orchiectomy. These recordings were found to parallel the clinical features of each individual. In many patients a return of the carcinoma was signalled by a rise in the serum alkaline phosphatase before digital examination revealed any evidence of recurrent prostatic changes. Stilbestrol administered to this group of patients was found to be of value in the control of their symptoms but not of the disease.—J. L. M.

Teratoma of the Testis. Report of Sixty-Five Cases. Barner, J. L. *Am. J. Roentgenol.*, **54**:257-261. 1945.

Sixty-five cases of teratoma of the testis have been observed and treated within the past 33 months at an Army general hospital. The average age at the time of discovery of the lesion was 28 years. The most important diagnostic sign is painless swelling of the testicle. Three-fourths of the patients received medical attention within the first year after symptoms were noted. Treatment consisted of orchidectomy, removal of the cord high at the internal abdominal ring, and postoperative irradiation. Seven of the 65 patients have died, the same number have had continuing symptoms. The remainder are believed to be well and to have returned to a wage-earning, civilian life.—E. H. Q.

Interstitial Cell Tumors of the Testicle. Ranstrom, S. [Upsala Univ., Upsala, Sweden] *Acta path. et microbiol. Scandinav.*, **22**:80-88. 1945.

A report of a case with photograph and photomicrographs, and a review of 14 cases from the literature. In the present patient, as in most of the other instances of the disease reported in adults, there were no signs of endocrine dysfunction.—M. H. P.

Cancer of the Prostate (A Clinical Study). Gonzalez, E. R. [Concepcion, Chile] *Urol. & Cutan. Rev.*, **49**: 473-489. 1945.

A review of the subject together with the author's clinical experiences.—V. F. M.

Urinary System

Squamous Cell Carcinoma of the Renal Pelvis. Kickham, C. J. E., and Stanton, R. H. [St. Elizabeth's Hosp., Boston, Mass.] *Am. J. Surg.*, **69**:249-252. 1945.

Report of a case of squamous cell carcinoma of the renal pelvis occurring in a 58 year old woman. This lesion was associated with a large calculus of the left kidney and a palpable left upper quadrant mass, but presented no urinary tract symptoms.—W. A. B.

Unusual Metastasis from a Primary Hypernephroma. Schrag, A. R., and Jordan, F. B. [Provincial Mental Inst., Edmonton, Canada] *Canad. M. A. J.*, **53**:168-169. 1945.

The case is reported because two different types of tumor were present at the same time—a hypernephroma of the kidney and an ulcerating adenocarcinoma of the stomach, and because there was metastasis of the hypernephroma to the tongue.—M. E. H.

Renal Tumors Simulating Gastrointestinal Disease. Lubash, S. [Beth Israel Hosp., New York, N. Y.] *N. Y. State J. M.*, **45**:45-51. 1945.

A study was made of all personal and service cases that had been seen at the Beth Israel Hospital for the past 5 years, either on the urological or other services. From these groups, 33 patients were found, upon pathologic study, to have renal tumors. The oldest patient was 75; the youngest was 38; the average age was 54. There were 22 men and 11 women. Gastrointestinal symptoms were presented in 23 cases (69%) of the entire series. This also includes symptoms referable to the gall bladder.

Concerning the tumors, hypernephromas were the most common (22 cases, or about 65%). Next in frequency were tumors of the renal pelvis (6 cases, or 17%). Of these growths 4 were malignant (carcinoma) and 2 nonmalignant (benign papilloma or fibroepitheliomas). There were 3 cases of carcinomas of the kidney (or 8.5%), one of lipoma of the kidney, and one of unrecognized pathology. Five cases (or about 15%) were masked by symptoms other than urologic and mimicked such diseases as carcinoma of the ovary, carcinoma of the transverse colon, possible gall bladder or liver malignancy, acute suppurative cholecystitis, that exploratory surgery was indicated. In none of these 5 patients was there at any time an indication of urological disease. Urines in all were practically negative; an occasional white blood cell was noted in 3. All 3 cases of carcinoma were included in this group. The other 2 were hypernephromas. Histories on 15 cases are reported.—J. L. M.

Wilms' Tumors. Dean, A. L. [Memorial Hosp., New York, N. Y.] *N. Y. State J. M.*, **45**:1213-1217. 1945.

The author presents a general discussion of Wilms' tumors including incidence, morphology, symptoms, diagnosis, differential diagnosis, and treatment.—J. L. M.

Papillomas of the Verumontanum. Honke, E. M. *J. Iowa M. Soc.*, **35**:427-428. 1945.

Case report. Papillomas located in the posterior urethra are usually preceded by inflammation. Although these growths may be benign at first they often become malignant later, as do papillomas of the bladder. Examination of the posterior urethra is of importance since symptoms frequently occur sufficiently early to allow removal of the growth before malignant change takes place.—M. E. H.

Some Surgical Aspects of Urinary Bilharziasis. Ward, R. O. *Proc. Roy. Soc. Med.*, **39**:27-38. 1945.

A well illustrated account of this disease, containing a section on neoplasms of the bladder in Egypt is presented. In 2 years at the Kasr-el-Aini Hospital, Cairo, of 130 cases of carcinoma of the bladder 114 were of the papilliferous type, and the great majority of these were judged to be the result of bilharzial disease. Twenty-two occurred in patients under the age of 30. Ten of the cancers were of the nodular infiltrating type.—E. L. K.

Endometrioma of the Urinary Bladder: Report of Five Cases. Balfour, D. C., Jr. *Proc. Staff Meet., Mayo Clin.*, **20**:129-133. 1945.

Forty-six cases of this unusual form of endometriosis have previously been reviewed in the literature since it was first described as an entity in 1921. In 42 of the 46 cases there had been previous pelvic surgery or associated pelvic disease.

In the past year 5 cases of endometrioma of the bladder were seen at the Mayo Clinic, and these are reported. All 5 had undergone pelvic operation previously or had associated pelvic disease, and all patients showed some urinary symptoms. Cystoscopic examination aided in the diagnosis of two of these cases; in a third the diagnosis was confused with inflammation and carcinoma.—J. L. M.

Oral Cavity and Upper Respiratory Tract

Malignant Tumors of the Nasal Cavity: Report Based on Sixteen Cases in which a Fronto-Ethmoid Approach was Employed. Havens, F. Z., and Thornell, W. C. *Proc. Staff Meet., Mayo Clin.*, **20**:125-128. 1945.

The authors believe that the operation described is valuable in cases of malignant tumor of the nasal cavity in which there is no roentgenographic evidence of involvement of bone. This method of treatment of malignant tumors of the nasal cavity was used in 16 patients during the period from January 1934 to July 1944. Fourteen (88%) of these patients remained free from any evidence of recurrence to the time this paper was written. Two representative case reports are presented in detail.—J. L. M.

Transitional Epithelial Cell Carcinoma of the Nasopharynx. Whiteleather, J. E. [Baptist Memorial Hosp., Memphis, Tenn.] *Am. J. Roentgenol.*, **54**:357-369. 1945.

After a historical and clinical-pathological presentation of the subject, the author reports 16 cases followed for periods of 1 to 24 months. All the patients had roentgen therapy, usually 2,100 to 3,200 r to the primary lesion (tumor dose) and additional treatment to any involved nodes. Three patients are living with disease and 3 apparently free from disease, but the follow-up period is too short for any conclusions.—E. H. Q.

Cancer of the Mouth: Its Present Day Treatment. Mayne, W. [Cook Co. Hosp., and Univ. of Illinois Dent. Sch., Chicago, Ill.] *Am. J. M. Sc.*, **210**:548-554. 1945.

A review.—J. G. K.

Parotid

Congenital Hemangioma of the Parotid Gland. Glaser, K., Mehn, W. H., and Schultz, L. W. [Children's Memorial Hosp. and Otho S. A. Sprague Memorial Inst., Chicago, Ill.] *J. Pediat.*, **28**:729-732. 1946.

This case is reported because the authors feel that certain important conclusions can be drawn from their observations. The contrast between the benign appearance of the tumor both macroscopically and microscopically were in sharp contrast to the highly malignant clinical course. The treatment recommended is the total extirpation of the growth, sacrificing the facial nerve and parts of the parotid gland if necessary. Surgery should be followed by radiation therapy. Prognosis is favorable where surgery has been early and radical.—M. E. H.

Intrathoracic Tumors

Presternal Cyst. Report of a Case. Seybold, W. D., and Clagett, O. T. [Mayo Foundation and Mayo Clin., Rochester, Minn.] *J. Thoracic Surg.*, **14**:217-220. 1945.

The cyst resembled a bronchogenic one, which is well recognized as occurring in the mediastinum, but this was situated in the subcutaneous tissues at the sternal angle. It was thin-walled, lined by ciliated pseudocolumnar epithelium, and mucous glands, cartilage, vessels, and nerves were recognized in the wall. The mass had been present in this 55 year old woman as long as she could recall. In recent years a sinus discharging small amounts of thin milky fluid had appeared over it. The cyst and sinus were removed without untoward event.—E. E. S.

Gastrointestinal Tract

Gastric Adenomas: A Pathologic Study. Rienits, J. H., and Broders, A. C. [Mayo Foundation, Rochester, Minn.] *West. J. Surg.*, **54**:65-69. 1946.

This is the final division of a series of papers by the authors appearing currently in the *West. J. Surgery* concerning a pathological study of gastric adenomas. The collected divisions will eventually be published as a monograph. This present manuscript deals with certain general comparisons of the various series studied. Certain observations made and certain conclusions reached deserving special attention are emphasized. Size, as measured by diameter, the volume of the tumor, and the thickness of the involved mucosa seemed to bear a relation to the malignant changes present. If a gastric adenoma has a diameter greater than 2.3 cm., or if the tumor mucosa external to the muscularis mucosa is thicker than 0.44 cm., or if the calculated volume of the tumor is greater than 4.15 cc., the tumor is very probably malignant. However, some of the adenomas smaller than this harbored adenocarcinomas of grades 1, 2 or 3. The management of a patient presenting a gastric tumor depends on: the age

and general condition of the patient, presence of metastasis, the size, location and number of the adenomas, the presence of symptoms, complications and technical difficulties.—M. E. H.

Carcinoma of the Stomach with Multiple Annular Metastatic Intestinal Infiltrations. HELLER, E. L. [Pittsburgh, Pa.] *Arch. Path.*, **40**:392-394. 1945.

Report of a case.—J. G. K.

LIVER

Primary Carcinoma of the Liver. FEASBY, W. R. [Toronto West. Hosp., Toronto, Canada] *Canad. M. A. J.*, **53**:486-488. 1945.

A case report with autopsy findings. The tumor was of the mixed cell type, both liver parenchyma cells and bile duct cells being present. The polythrombocythemia present led to the correct ante mortem diagnosis of primary carcinoma of the liver.—M. E. H.

Multiple Bile Duct Adenoma. BLEYER, L. F. [Chicago Pathological Society, Regular Meeting, April 8, 1946] *Proc. Inst. Med. Chicago*, **16**:205. 1946.

Case report. The patient gave a clinical history suggestive of gastric ulcer. The x-ray findings corroborated this. Exploratory laparotomy revealed millet-like nodules in both lobes of the liver which proved to be bile duct adenomas on biopsy.—M. E. H.

Primary Carcinoma of the Liver. WEBB, A. C. [Provident Hosp., Chicago, Ill.] *Arch. Path.*, **40**:382-386. 1945.

Discussion of 12 cases.—J. G. K.

Primary Carcinoma of the Liver in Infants and Children. ROSENBLATT, M. G., and MAY, J. A. [Portland, Ore.] *Northwest Med.*, **45**:96-97. 1946.

Two cases are reported, occurring in males aged 4 months and 2½ years respectively. The usual symptoms are enlargement of the liver, abdominal distress, anemia, and cachexia. Jaundice, which was present in both of these children, has been inconstant in appearance or a terminal manifestation in other reported cases. Despite its rarity, primary liver cell carcinoma is the most common hepatic tumor of infancy and to a lesser degree of childhood.—H. S. K.

SPLEEN

Primary Splenic Neoplasm. BOSTICK, W. L. [Univ. of California Med. Sch., San Francisco, Calif.] *Am. J. Path.*, **21**:1143-1165. 1945.

A review and a classification of splenic neoplasms are presented, together with reports of 7 instances of primary reticulolymphosarcoma, reticulum-cell sarcoma, primary endothelioma (2 cases), hemangioma, lymphangioma, and epidermoid cyst, respectively.—J. G. K.

Adrenal Neuroblastoma. OGILVIE, T. A. *Brit. J. Surg.*, **32**:78-83. 1944.

An account of a case with a review of the literature.—E. L. K.

BONE AND BONE MARROW

Interscapulothoracic Disarticulation of the Arm. BERMAN, J. K. [Indiana Univ. Sch. of Med., Indianapolis, Ind.] *Surgery*, **18**:256-266. 1945.

This paper describes an operative technic indicated in the treatment of: (1) carcinoma or sarcoma of the hand, forearm or arm, with metastases to the axilla and involvement of the shoulder joint or shoulder muscles; (2) axillary tumors adherent to vessels and nerves; and (3) extensive irreparable trauma to the extremity. The 5 patients in this series upon whom this procedure was carried out survived from 7 months to 1 year and 7 months; of these, 3 who had sarcoma died of metastases, but the remaining 2 were alive and apparently well at 18 and 19 months after operation.—W. A. B.

Consideration of Some of the Difficulties in Diagnosis and Treatment of Osteogenic Sarcoma and Chondrosarcoma. EDITORIAL. *Penn. Med. J.*, **48**:817-819. 1945.

Diagnosis of the pathologic type of malignant bone tumor cannot be made with certainty from the roentgen examination alone. Benign conditions may simulate malignant bone tumors. In order to make as many correct diagnoses as possible, one should do a complete skeletal survey in every patient where a bone lesion is suspected of being malignant, and the material submitted to the pathologist as a biopsy should be representative of the tumor. Adequate time should be allowed to make and study paraffin sections if there is any doubt about the nature of the lesions.—J. L. M.

Eosinophilic Granuloma of Bone. MICHAEL, P., and NORCROSS, N. C. [U.S.N.R.] *U. S. Nav. M. Bull.*, **45**:661-668. 1945.

The paper gives a brief review of this benign, destructive lesion, first described as a clinical entity in 1940, together with reports of 2 cases that occurred in naval personnel. It includes roentgenograms and photomicrographs. Treatment is excision and x-ray therapy.—C. W.

Plasmacytoma with Amyloidosis. Report of a Case with Postmortem Findings. BLUMBERG, N., and FISHBACK, M. W. [Philadelphia, Pa.] *M. Rec.*, **158**:281-284. 1945.

Pain in the back, developing after a short period of malaise, became excrutiating, but x-ray just before admission to the hospital revealed only "arthritis." Later a compression fracture of a vertebra was detected. The ribs, sternum, and ilium shared in the tenderness, and erythrocytes and casts appeared in the urine. Bence-Jones proteinemia could not be demonstrated, but rouleau formation was excessive in a blood smear. Ninety-five per cent of intravenous congo red dye was absorbed. The diagnosis was established by biopsy of a rib, and autopsy showed that in addition to plasmacytoma in many portions of the skeleton, mesenteric lymph node, and in the lung, there was a deposit of amyloid in the kidneys, liver, and spleen. A review of some of the literature concerning myeloma is appended. This is said to be the 41st case to be reported having a coincidence of amyloid and myeloma. There are no photomicrographs.—E. E. S.

Giant-Cell Tumor of Bone in a Four-Month Old Infant. PROFFITT, W. E., and WYATT, O. S. [Minneapolis, Minn.] *J. Lancet*, **66**:163-165. 1946.

Case report. The only absolute diagnostic criterion is biopsy. The treatment remains as Bloodgood outlined it in 1910 and has been considered until recently too radical. Giant-cell tumors are benign in nature.—M. E. H.

Extradural Diploic Epidermoids Producing Unilateral Exophthalmos. Thornhill, E. H., and Anderson, B. [Duke Hosp. and Med. Sch., Durham, N. C.] *Am. J. Ophth.*, **27**:477-483. 1944.

Extradural diploic epidermoids (apparently a cholesteatoma), producing unilateral exophthalmos and gradual loss of vision in a young woman were completely removed through the supraorbital approach. Photographs, roentgenograms, and a review of the literature are presented.—M. H. P.

Blood Vessels

Haemangioma Pontis. Altschul, R. [Sch. of Med. Sc., Univ. of Saskatchewan, Saskatoon, Canada] *Canad. M. A. J.*, **53**:465-467. 1945.

A case is reported and a tentative explanation of the morphogenesis of this condition is presented.—M. E. H.

The Clinical Significance of Hemangiomata of the Leg. Haynes, B. W., Jr. [Med. Coll. of Virginia, Richmond, Va.] *Virginia M. Monthly*, **73**:221-223. 1946.

Case report. Hemangiomas of the leg are important clinically, chiefly from the standpoint of the pathological physiology they engender in the cardiovascular system. Growth disturbances of the extremity and osteohypertrophy may accompany the lesion. Ligation and excision of the vessels connecting the mass to the general circulation is the treatment of choice; selected cases may require complete excision of the tumor.—M. E. H.

Vertebral Hemangioma in Children. Kaplan, I. [Bellevue Hosp., New York Univ. Coll. of Med., New York, N. Y.] *J. Pediat.*, **28**:498-502. 1946.

During the past two decades, only 5 cases of bone hemangioma have been referred to the Radiation Therapy Department at Bellevue Hospital and the present case report is the only one in a child with vertebral involvement. Irradiation is the treatment of choice and the patient presented shows the favorable results of this type of therapy.—M. E. H.

Über die bösartigen Hämangiome in der Milz und Leber. [Malignant Hemangiomas in the Spleen and Liver.] Siirala, U., and Näätänen, E. [Helsingfors Univ., Helsingfors, Finland] *Acta path. et microbiol. Scandinav.*, **17**: 453-465. 1940.

A report of a case in a 20 year old woman. The hemangioma in the spleen was apparently primary, and that in the liver, metastatic.—M. H. P.

Leukemia, Lymphosarcoma, Hodgkin's Disease

Chronic Myelogenous Leukemia in Infancy. Keith, H. M. *Proc. Staff Meet., Mayo Clin.*, **19**:581-582. 1944.

The author reports a case of chronic myelogenous leukemia in which the onset of the disease occurred before the patient was three months of age. The disease lasted for twelve months, and was terminated by the death of the patient.—J. L. M.

Coexistence d'une leucémie lymphoide et d'un carcinome. [Coexistence of Lymphoid Leukemia and Carcinoma.] Delcourt, R., and van Vliet, P. [Brussels, Belgium] *Acta med. Scandinav.*, **119**:47-56. 1944.

In the cases reported, leukemia and adenocarcinoma, apparently arising from the biliary passages, developed independently of one another.—M. H. P.

Case of Leukemic Myelomatosis (Plasma Cell Leukemia). Søeborg-Ohlsen, A., and Nielsen, O. P. [St. Elizabeth's Hosp., and Copenhagen County Hosp., Copenhagen, Denmark] *Acta med. Scandinav.*, **122**:271-279. 1945.

A case of leukemic myelomatosis is presented. Its clinical picture corresponded to an acute leukemic state. Differential counts on the circulating blood and bone marrow showed a considerable preponderance of myeloma cells. Plasmaglobulin was not augmented. The myeloma cells are less basophilic than normal plasma cells.—Authors' summary. (G. H. H.)

The Value of Penicillin in the Control of Sepsis Complicating a Case of Severe Granulocytopenia (Aleukemic Leukemia). Kugel, V. H., and Schnitker, M. A. *Ann. Int. Med.*, **23**:1001-1006. 1945.

A case report. Penicillin controlled bacterial infection of the mucous membranes during attacks of granulocytopenia in a patient with aleukemic leukemia, but there was no evidence that the drug affected the leukemia *per se*.—J. G. K.

Follicular Lymphoma (Brill-Symmers Disease) Unsuccessfully Treated with Penicillin: A Case Report. Drey, N. W., and Queen, F. B. [Brigham City, Utah] *Ann. Int. Med.*, **23**:1009-1015. 1945.

More than 11,000,000 units of penicillin were given intramuscularly over a 60 day period. There was no change in the clinical course, and biopsies of the lymph nodes before and after penicillin therapy revealed no change in the histopathological characteristics of the lesion.—J. G. K.

Leukemia Complicated by Cancer. Berk, M., and Movitt, E. R. [Veterans' Administration Facility, San Francisco, Calif.] *Am. J. Clin. Path.*, **15**:246-249. 1945.

Lymphogenous leukemia as proved by blood examinations and by biopsy of a cervical lymph node, and a squamous cell carcinoma of the larynx with metastasis to an adjacent lymph node were found in a 71 year old white man.—J. G. K.

Chloroma—A Clinico-Pathologic Study of Two Cases. Goodman, E. G., and Iverson, L. [Duke Univ. Sch. of Med., Durham, N. C.] *Am. J. M. Sc.*, **211**:205-215. 1946.

Chloroma represents a rare form of myeloblastic leukemia in which the cells show an unusual tendency toward tumor formation. Spectroscopic and polariscopic studies suggest that the green pigment is an intermediary product in the breakdown of hemoglobin to bilirubin.—M. T.

Two Cases of Hemolytic Anemia with Leukemoid Reaction of the Myeloid Type. Arey, S. L. [Minneapolis, Minn.] *J. Lancet*, **66**:166-167. 1946.

Leukemoid reactions of the lymphatic type are seen commonly in infectious mononucleosis and in pertussis. Two cases are presented in which during hemolytic crises the peripheral blood picture closely resembled that of myelogenous leukemia.—M. E. H.

The Surgical Problem of Cancer in Lymph Nodes. Sugarbaker, E. D. [Ellis Fischel State Cancer Hosp., Columbia, Mo.] *Surgery*, **18**:608-619. 1945.

When clinical demonstration of node involvement exists,

the course generally accepted is *en bloc* removal of the node groups. The proper method of dealing with draining node areas in which there is no clinically demonstrable disease is in dispute; some recommend prophylactic dissection whenever the tumor is of known metastasizing potentialities, and others feel that careful follow-up studies, with avoidance of unnecessary surgery is the procedure of choice.—W. A. B.

Hodgkin's Disease. Goldman, L. B., and Victor, A. W. [Dept. of Hosps., City of New York, N. Y.] *N. Y. State J. M.*, **45**:1313-1318. 1945.

The authors reported a statistical analysis of 212 cases of histologically proven Hodgkin's disease (*J.A.M.A.*, **114**: 1611. 1940). Since that date their study has continued with an additional 107 cases, a total of 319 patients. In the previous paper the disease with its different manifestations was described in detail; in this paper they emphasize the salient clinical features and stress the relative value of the various methods of treatment. In this present series 193 patients (60%) were observed until death and 69 of these (32%) had complete postmortem studies. The following aspects of Hodgkin's disease are discussed: history and physical findings, sternal node involvement, cutaneous manifestations, thoracic involvement, gastrointestinal involvement, abdominal localization, bone involvement, blood and marrow picture, the effect of pregnancy, and finally treatment.—J. L. M.

Uveitis Associated with Hodgkin's Disease. Report of a Case. Kamellin, S. [Northwestern Univ. Med. Sch., Chicago, Ill.] *Arch. Opth.*, **31**:517-519. 1944.

The association of uveitis with Hodgkin's disease has not been reported previously. This is the report of one case. Whether the uveitis that accompanied Hodgkin's disease in this case is due to the latter condition, or is a concomitant disease is not clear. The pathologic section of a cervical lymph node, by means of which the diagnosis of Hodgkin's disease was established, and colored drawings of the lesions in the ocular fundi are well reproduced.—E. C. R.

An Unusual Case of Hodgkin's Disease. Second Report. Avery, J. W., and Warren, J. W. [Hollywood, Calif.] *Arch. Ophth.*, **34**:318. 1945.

At the age of 50, a woman with Hodgkin's disease in the early stages suffered involvement of the lymphatics in the bulbar conjunctiva of each eye. These were flat, reddish growths that were excised but reappeared in the same locations within 6 months. Roentgen radiation was applied, and at the present time, 5 years later, there is no evidence of recurrence.—E. C. R.

Hodgkin's Disease: Special Reference to Survival. Smeltzer, C. C. [Knoxville, Tenn.] *J. Tennessee M. A.*, **38**:281-284. 1945.

The introduction includes a brief summary of the characteristics of the disease as listed in textbooks. Report is made of the survival times given in several large series. Marked extremes in length of life after onset have been noted by many observers regardless of therapy. Two cases are presented as illustration of the variability of course; one patient who developed enlarged cervical nodes was dead 10 weeks later, another patient has lived 13 years after removal of an affected node followed by radiotherapy. Recurrence in the latter case occurred 1 year ago and was treated as before.—E. E. S.

Binocular Papilledema in a Case of Torulosis Associated with Hodgkin's Disease. Cohen, M. [New York, N. Y.] *Arch. Ophth.*, **32**:477-479. 1944.

Report of an unusual case in which Hodgkin's disease was associated with torulosis. The outstanding clinical features were pronounced binocular papilledema, and severe cerebral symptoms. Torula organisms were recovered from the spinal fluid, and were also found in brain tissue at autopsy.—E. C. R.

Adrenal

Hypertensive Retinopathy Associated with Adrenal Medullary Tumor (Pheochromocytoma). Rodin, F. H. [Mount Zion Hosp., San Francisco, Calif.] *Arch. Ophth.*, **34**:402-407. 1945.

Pheochromocytoma is a rare form of chromaffin tumor, the cells of which contain epinephrine, or an epinephrine-like substance. A patient with this type of tumor of the adrenal medulla showed changes in the retinas characterized by alterations in the normal appearance of the blood vessels, hemorrhages, and the formation of exudate. The appearance of the ocular fundi returned to normal after surgical removal of the tumor. Fundus photographs before and after operation are included. This is a new clinical entity.—E. C. R.

Pancreas

Case Report of Barnes Hospital. Clinical and Postmortem Records Used in Weekly Clinicopathologic Conference at Barnes Hospital, St. Louis. Wood, W. B., Jr., and Moore, R. A., Editors. *J. Missouri M. A.*, **43**:391-396. 1946.

Case 89 proved to be one of carcinoma of the islands of Langerhans with extensive metastases to the lungs, pleurae, kidneys, tissues of the anterior mediastinum, the mediastinal bronchial periaortic, periportal, peripancreatic and pelvic lymph nodes, skull, dura, leptomeninges, ribs and vertebrae. Exsanguinating hemorrhage from a chronic ulcer at the esophageal-cardiac junction of the stomach was the immediate cause of death.—M. E. H.

Clinical Pathological Conference. Lund, P. K. Swedish Hosp., Seattle, Wash.] *West. J. Surg.*, **54**:162-165. 1946.

The case was one of adenocarcinoma of the pancreas arising in Wirsung's duct and causing obstructive jaundice and subacute cholecystitis. The discussion centers around the errors in clinical diagnosis and the necessity for careful evaluation of clinical laboratory procedures.—M. E. H.

Islet-Cell Tumors of the Pancreas. Maxeiner, S. R., and Bundy, H. E. [United States Veterans Hosp., Minneapolis, Minn.] *Surgery*, **18**:171-177. 1945.

The literature is reviewed and Whipple's triad for diagnosis of islet-cell tumors cited. A case is presented that illustrates all of these points: (1) attacks of insulin shock during fasting or due to an over-fatigued state, (2) blood sugar findings of 50 mgm.% or less, and (3) prompt relief by the ingestion of glucose. In the patient

presented, no tumor was palpable at laparotomy, but following the removal of 75% of the pancreas (that portion to the left of the superior mesenteric vessels), all symptoms were relieved. In the resected area, several small nodular masses were found on serial section, and these proved to be typical islet-cell tumors.—W. A. B.

Hypophysis

Slow-Growing Hypophyseal Tumor Associated with Hypothyroidism—A Case Report. Swartz, H. [Fort Dix, N. J.] *N. Y. State J. M.*, **45**:1683-1684. 1945.

A case of slow-growing pituitary tumor associated with hypothyroidism is presented. The tumor was unusually large. Its relationship to thyroid deficiency is discussed. The importance of considering the endocrine glands as anatomic units of a single interplaying physiologic system is pointed out.—J. L. M.

Thymus

Tumors of the Thymus in Myasthenia Gravis. Murray, N. A., and McDonald, J. R. [Mayo Foundation and Mayo Clinic, Rochester, Minn.] *Am. J. Clin. Path.*, **15**:87-94. 1945.

Fifteen cases of tumors of the thymus associated with myasthenia gravis were studied. Most of the growths were of a single histologic type being comprised of lymphocytes and larger pale cells with faintly acidophilic cytoplasm and occasional Hassal's corpuscles. Metastases were noted in 2 instances and direct extension into the vena cava in 1 case. The authors conclude that the incidence of myasthenia gravis among patients suffering from thymoma is nearly 100%.—J. G. K.

Thyroid

Metastatic Hypernephroma of the Thyroid. Long, G. C., and Black, B. M. *Proc. Staff Meet., Mayo Clin.*, **20**:43-48. 1945.

The authors report a case of hypernephroma with thyroid metastasis for which thyroidectomy was done. It was the third case observed at the Mayo Clinic over a period of fifty years, and the eleventh reported in the literature. The salient feature was the latent period of about eight years between the time of nephrectomy and the first sign of metastasis. It appears that metastatic carcinoma of the thyroid occurs most frequently in glands which clinically are thought to be adenomatous.—J. L. M.

Congenital Teratoma of the Thyroid. Munro, E. H., and Waldapfel, R. [St. Mary's Hosp., Grand Junction, Colo.] *Am. J. Surg.*, **64**:271-275. 1944.

Report of a case in a 4 week old male infant is presented. The tumor had been present at birth, and progressed in size until it caused conspicuous respiratory difficulty. Successful removal of the growth was performed when the infant was 6 weeks old. The nodular neoplasm was confined to the left lobe of the thyroid. All 3 germinal layers were represented in the tumor.—W. A. B.

Hürthle Cell Tumor of the Thyroid Gland in an Infant. Morrow, W. J. [Chicago, Ill.] *Arch. Path.*, **40**:387-391. 1945.

Case report.—J. G. K.

Miscellaneous

Cancer in Relation to Usages. Three New Types in India. Khanolkar, V. R., and Suryabai, B. [Bombay, India] *Arch. Path.*, **40**:351-361. 1945.

Three new types of cancer are found in different regions of India; namely, Bombay, Vizagapatam and Patna. They may be called the dhoti, chutta and khaini cancers. They are associated with the wearing of a light garment (the dhoti), smoking of a cigar (the chutta) with the lighted end in the mouth, and the depositing of tobacco and lime (khaini) behind the lower lip of the mouth. A histologic study of the lesions that precede the development of 2 of these cancers reveals a similarity in appearance between them and the "precancerous" stages in mice that have been painted experimentally with carcinogenic substances. The observations reported, and a review of the available experimental literature on the subject of changes in the skin as a result of exposure to mechanical and thermal irritants lead to the conclusion that in the induction of the tumors described the part played by the reaction of the tissues that are the seat of the cancer is equal in importance to that played by the carcinogenic substances themselves.—Authors' summary.—J. G. K.

Desmoid Tumor. Green, C. G. [Houston, Tex.] *Arch. Surg.*, **50**:304-306. 1945.

Report of a case in a 65 year old man. The tumor occurred on the lower part of the anterior abdominal wall, and histologically was diagnosed as a fibroma with sarcomatous changes (malignant desmoid).—W. A. B.

Coincidental Adenomas of Islet-Cells, Parathyroid Gland and Pituitary Gland. Shelburne, S. A., and McLaughlin, C. W. [(M.C.) U.S.N.R.] *J. Clin. Endocrinol.*, **5**:232-234. 1945.

A 26 year old man was observed to have hyperinsulinism until removal of an adenoma of the pancreatic islands of Langerhans. Development of renal stones and the recognition of supranormal levels of calcium in blood and urine led to the removal two years later of an adenoma of the parathyroid gland. The presence of a pituitary tumor was suggested by radiological evidence of erosion of the floor of the sella turcica and the clinoid processes and by defects of the temporal quadrant of the right visual field. The authors claim that their case and another cited in the literature represent a new clinical syndrome characterized by the occurrence simultaneously of adenomatous tumors of three endocrine glands, the pituitary, pancreas, and parathyroids.—J. B. H.

Symmetrical Nodular Lipomatosis. Sigurdson, L. A. [Univ. of Manitoba, Winnipeg, Canada] *Canad. M. A. J.*, **53**: 274-275. 1945.

This case is reported because of the large number of the lipomas. The tumors, varying from pea-size to orange-size, were on the arms, trunk, and thighs.—M. E. H.

Dupuytren's Contracture: Fibroma of the Palmar Fascia. Clay, R. C. [Johns Hopkins Hosp. and Sch. of Med., Baltimore, Md.] *Ann. Surg.*, **120**:224-231. 1944.

The purpose of this paper is to show from a study of 17 cases (15 in males and 2 in females) that Dupuytren's contracture is due to a neoplasm—a cellular fibroma of the

palmar fascia. The predominance in males and the positive family history given by 5 patients agrees with previously reported data.—M. H. P.

Infiltrating Benign Lipomas of the Extremities. Regan, J. M., Bickel, W. H., and Broders, A. C. [Mayo Clin., Rochester, Minn.] *West. J. Surg.*, **54**:87-93. 1946.

Two cases are reported that form the background of the discussion concerning differential diagnosis, malignant changes, origin of the tumors, and treatment.—M. E. H.

Tumors and Tumor Metastases in Their Relation to Trauma. Editorial. *Am. J. Roentgenol.*, **55**:213-214. 1946.

Literature is reviewed describing experiments that tend to demonstrate that trauma is not an active agent in determining the sites of tumor metastases.—E. H. Q.

Chylangioma Cavernosum Mesenterii. Lubitz, J. M., and Flynn, R. W. [U. S. Marine Hosp., Chicago, Ill.] *Surgery*. **18**:772-777. 1945.

Report of a case, with a review of 5 previously reported in the literature.—W. A. B.

STATISTICS

A Statistical Analysis of 1,214 Cases of Carcinoma. McPhee, J. G., and LaCroix, W. R. [Vancouver General Hosp., Vancouver, B. C., Canada] *Canad. M. A. J.*, **54**:573-584. 1946.

Of the total of 7,186 autopsies performed during the 10 year interval from 1934 to 1943, 1,214 were recorded as revealing definite evidence of malignant growth of some type. Eight hundred and seventy were males; 344 were females. A statistical analysis is presented according to sex, age at death, primary site of new growth, sites of metastases and the microscopic type of carcinoma.—M. E. H.

Observations on Malignant Disease in Ceylon Based on a Study of Two Thousand Two Hundred and Ninety-Five Biopsies of Malignant Tumors. Cooray, G. H. [Univ. of Ceylon, Ceylon] *Indian J. M. Research*, **32**:71. 1944.

In a preliminary investigation regarding malignant disease in Ceylon, an attempt has been made to make an analysis of the malignant tumors sent for histological examination from various hospitals on the island to the Department of Pathology of the University of Ceylon during the 7 year period from 1936 to 1942. The total number of specimens examined histologically was 10,880. While most of these were obtained at the time of operation, a few were postmortem specimens. The author includes postmortem material among biopsy reports. The specimens were obtained from Singhalese, Tamils, Burghers, Moors, Europeans and Malayans. Of 2,295 malignant tumors, 41 were malignant melanomas. The most common sites of the 1,815 primary carcinomas were cervix uteri, 316; buccal cavity, 196 (males) and 78 (females); penis, 248; breast, 5 (males) and 170 (females); skin, 175 (males) and 71 (females). There were 32 cancers of the corpus uteri and 35 chorionepitheliomas. The author remarks upon the high incidence of the latter tumor and states that hydatidiform mole occurs commonly among the women of Ceylon. Three cases of cancer of the penis occurred between the ages of 15 and 24 and some references are given to the literature dealing with the very high incidence of this form of cancer in Southern Asia. Sixty-one per cent of the skin cancers arose from chronic nonspecific ulcers whereas of the 23 malignant melanomas in men, all except one arose on the plantar surface of the foot, and of the 18 similar growths in women more than half arose on the same site. Eleven retinoblastomas were seen in children under 5.—E. L. K.

CANCER CONTROL AND PUBLIC HEALTH

Industrial Management and Occupational Cancer. Hueper, W. C. [Warner Inst. for Therapeutic Research, New York, N. Y.] *J. A. M. A.*, **131**:738-741. 1946.

The rapid increase in the number of recognized and suspected agents causing industrial cancers makes it more likely that new and heretofore unsuspected carcinogenic substances of an industrial nature will be discovered during the coming years. For this reason a nationwide survey by skilled investigators should be made to determine the actual scope of the problem of industrial cancer. Representatives of management, industrial physicians, members of the Public Health Service, and of departments of industrial hygiene, and possibly also representatives of the workers, should participate in such a survey.—M. E. H.

The Widening Horizon in Cancer Education and Treatment in Ontario. Crozier, L. J. [Victoria Hosp., London, Ont., Canada] *Canad. M. A. J.*, **54**:601-602. 1946.

The cancer campaign in Ontario coincided with those in Canada and the United States. It was threefold in purpose: for public education, for research, and for better cancer treatment facilities.—M. E. H.

Examination of the Breasts and Pelvic Organs in Apparently Well Women. Review of the Findings in 1,600 Women Examined at the Cancer Prevention Clinic. Webster, A., Phillips, M. A., Nadelhoffer, L., Oliver, M., and Parsons, E. [Chicago, Ill.] *Illinois M. J.*, **89**:239-241. 1946.

This is a review of the findings in 1,600 cases examined since May of 1943. Among the first 600 there were 10 proved cases of carcinoma of the breast. In the subsequent 1,000 no obvious carcinoma was found and of 126 women in whom biopsies were requested 11 were found with cervical erosions. Of the women on whom pelvic examinations were carried out, biopsy was advised in 13, and reports thus far have not indicated the presence of any cervical carcinoma. Uterine fibromyomas were discovered in 41 of these 1,000 women and in 16 surgery was indicated because of the size of the tumor. Since the examinations were carried out in apparently well women without complaints, the percentage of positive malignant growth is understandingly low.—M. E. H.

Cancer Control in Saskatchewan. Davison, R. O. [Regina, Sask., Canada] *Canad. J. Pub. Health*, **35**:150-153. 1944.

The Saskatchewan Cancer Commission is empowered by legislation passed in 1930 to collect data on mortality and treatment, to disseminate information to aid in control of the disease, to provide for the establishment of clinics for diagnosis and radiation therapy, to obtain a supply of radium, and to provide facilities for the diagnosis and treatment of cancer for all patients. During 1932 to 1942,

there were 8,897 patients (cancerous, "precancerous," and noncancerous) admitted to the clinics; these paid for services when they could, or the fees were paid by the municipality from which they came. At the 1944 session of the Saskatchewan Legislature, provision was made for care and treatment at the expense of the province for all patients who have resided there for at least 6 months immediately prior to application for admission to a clinic.—M. H. P.

Cancer Prevention Clinics. MacFarlane, C. [Philadelphia, Pa.] *Pennsylvania M. J.*, **48**:1348-1356. 1945.

The history of cancer prevention clinics is discussed. In a group of well women the author undertook to determine the value of periodic pelvic examinations in detecting cancer of the uterus in an early and curable stage or in the detection of inflammatory lesions of the cervix which are commonly believed to predispose to the development of cancer. Of 1,319 white women between the ages of 30 to 80 years and all presumably well, 550 have come regularly for routine pelvic examination twice a year while an additional 121 were somewhat irregular in their visits. Thus a total of 671 volunteers have been examined for a 5 year period.

Up until December 31, 1944, 10,318 visits were made, during the course of which the examining physicians discovered 11 cancers. Six of these were pelvic growths, and 3 were breast tumors. There was 1 cancer of the parotid gland and 1 of the skin. Eleven other cancers developed in these women during this observation period and were reported to the clinic; 2 of these were in the pelvis, 1 was in the hip, 1 in the pancreas, 1 in the lung, 1 hypernephroma, 1 lymphosarcoma, and 4 malignant tumors of the colon. In addition to the pelvic cancers, 832 benign lesions of the pelvic organs were discovered.

The establishment of similar clinics in Philadelphia in 1944 is described. In the first 6 months, 90 examinations were performed, and 9 cancers found. About 40% of the examinees were referred to the family doctor, and half of these physicians failed to reply.—J. L. M.

The Organization and Administration of a Gynecologic Tumor Clinic. Beecham, C. R., and Montgomery, T. L. [Temple Univ., Philadelphia, Pa.] *Pennsylvania M. J.*, **48**:697-700. 1945.

The authors give a description of what may be accomplished when the fields of gynecology and radiology pool their resources.—J. L. M.

Correction

Volume **6**:521 (Abstracts). 1946. **Thymonucleic Acids in Human Tumors.** Title should be: **"Nucleic Acids in Human Tumors."**

Index to Volume 6

Original Articles and Abstracts

Author and subject entries are included in one alphabet. Asterisk (*) indicates original article published in *Cancer Research*. Double asterisks (**) indicate papers read before the American Association for Cancer Research, Inc. Articles not otherwise designated are abstracts.

Book reviews are indexed under **Book Reviews,** and under name of author of book.

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EWING, J. Neoplastic Diseases. A Treatise on Tumors. Fourth edition, Philadelphia & London: W. B. Saunders Company. 1940, p. 449.

SAPHIR, O., and PARKER, M. L. Intracystic Papilloma of the Breast. Am. J. Path., **16**:189-210. 1940.

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